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**Studies on the Structure, Development, and
Physiology of the Nephridia of Oligochaeta.**

**III. The Branching and Division of Nephridia,
and Eisen's so-called 'safety valves'
in Pontoscolex.**

By

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With 10 Text-figures.

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1. INTRODUCTION.

THE earthworm *Pontoscolex corethrurus*, F. Muller, is the only representative of the sub-family Glossoscolecinae (Family Glossoscolecidae) in India, where it is confined to South India, Ceylon, and the Deccan plateau, being entirely absent from Northern India. The nephridial system of this worm has been studied by several well-known workers, but their accounts show many discrepancies, errors, and omissions; further, they have all missed the most important fact that the nephridia of this worm exhibit the phenomena of branching and division and are 'actually caught in the act of dividing up' (10). In fact, the nephridia of the different regions of the body of *Pontoscolex* form a graded series of stages showing how a large holonephridium, like that of *Lumbricus*, branches and divides to form several meronephridia like those of *Pheretima* (1) or *Eutyphoeus* (Part I).

Perrier (8) was the first to describe the nephridia of this genus, but his diagram of a nephridium from a post-clitellar segment is far from being accurate; his diagram of the anteriormost tufted nephridia of the second segment, which he called 'Glande à mucosité', is, however, more or less correct in outline, but neither he nor any of the subsequent workers has correctly interpreted the structure of this so-called 'gland'. Beddard (5) pointed out that the so-called 'Glande à mucosité' was 'a nephridium,¹ possibly formed by the fusion of two or three embryonic nephridia'. Although Beddard rightly recognized the nephridial character of this so-called gland, he failed to recognize that each nephridium is really composed of about 80 to 100 minute meronephridia, formed by the branching and division of a single nephridium, and not by the fusion of two or three embryonic nephridia. Beddard further stated that each of these two nephridia opened into the body-cavity by three funnels—another unfortunate mistake. Eisen (7) gave an outline diagram of a nephridium from the post-clitellar region, but he could not trace the course of the nephridial canal, as he found it too complicated; his diagram and description are also not quite accurate. Stephenson (9) described the position of the septa and the nephridia of the anterior segments, and stated that the anteriormost tufted nephridium opened into the hinder part of the pharynx, although both Perrier and Beddard had correctly found that it opened on the surface of the body. As regards the nephridia of the third, fourth, and fifth segments, Stephenson 'could not separate the posterior ends of these three nephridia', and could not say 'whether they interlace, or communicate' with one another; in fact, they neither interlace nor communicate, but are discrete and independent of one another.

Eisen (7) describes a number of longitudinal canals throughout the length of the typhlosole, which probably serve as 'safety valves', enabling the typhlosole to discharge superfluous blood into the intestine²—a rather surprising statement, which has been examined and found incorrect.

I am very thankful to Mr. K. Bhaskaran Nair of the Maha-

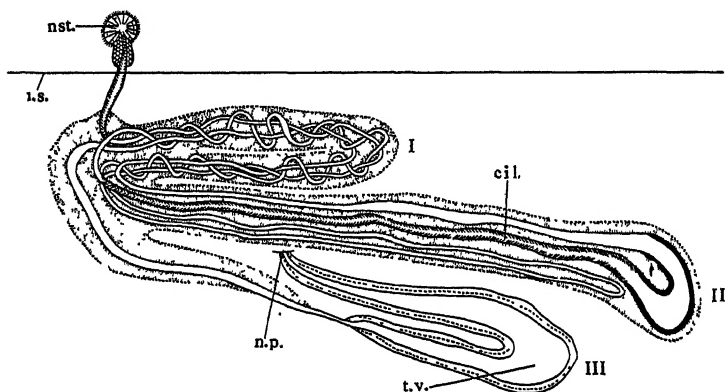
¹ The spaced word is mine.

² The spaced words are mine.

raja's College, Trivandrum (Travancore), who kindly sent me well-fixed specimens of this worm.

2. THE HOLONEPHRIDIA OF THE POST-CLITELLAR SEGMENTS.

The typical holonephridia are found in the post-clitellar region



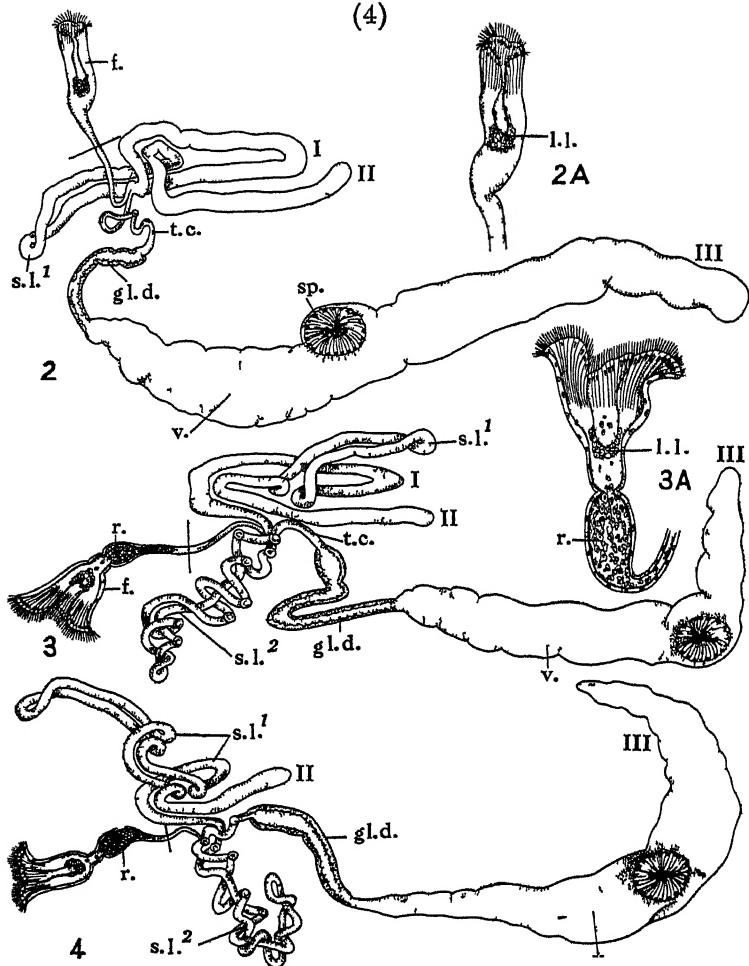
TEXT-FIG. 1.

A nephridium of *Lumbricus* (diagrammatic). *cil*, ciliated part of the intra-cellular canal; *is*, intersegmental septum; *np*, nephridiopore; *nst*, nephridiostome; *tv*, terminal vesicle; I-III, the three main loops. (From Meisenheimer, after Maziariski.)

of the body extending from the 24th or 25th to the last segment.¹ Each of these segments possesses a pair of holonephridia which resemble those of *Lumbricus* in general outline, but present a few important differences. Text-fig. 2 shows a post-clitellar nephridium of *Pontoscolex* and Text-fig. 1 that of *Lumbricus* for comparison. In *Pontoscolex* the pre-septal funnel (Text-fig. 2 A) is elongated and vase-shaped in appearance and is comparatively very large, and Eisen (7) is no doubt right when he says that 'the nephrostome is larger than I have seen in any other nephridium'. It measures about 300 μ in length and about 88 μ in diameter. The funnel is followed by a short chamber or 'receptacle' which is inconspicuous in the post-

¹ The total number of segments in the worm is 90 to 212.

(4)



TEXT-FIGS 2-4.

•Nephridia of the different regions of *Pontoscolex corethrurus* (semi diagrammatic).

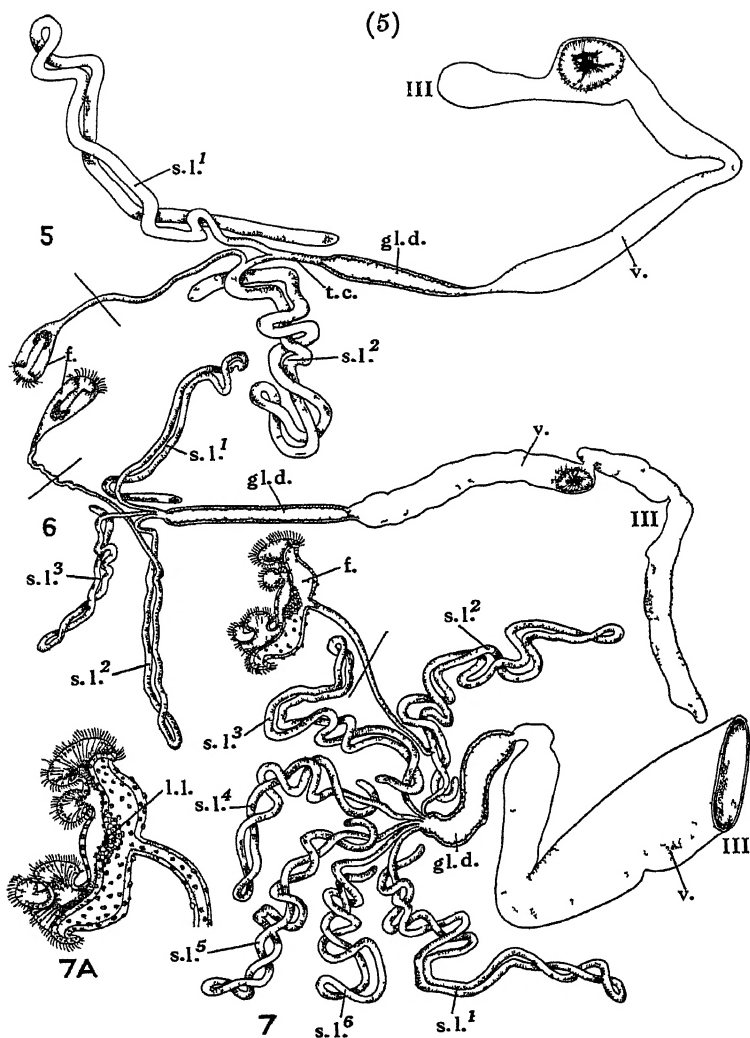
Fig. 2.—A nephridium from a post-clitellar segment.

Fig. 3.—A nephridium from a clitellar segment.

Fig. 4.—A nephridium of the 15th (last pre-clitellar) segment

In Figs. 2 and 3, a magnified view of the funnel is shown on one side. *f*, funnel; *gld*, glandular duct, *ll*, lower lip, *r*, receptacle filled with disintegrating coelomic corpuscles; *sl*¹ and *sl*², first and second subsidiary loops; *sp*, sphincter around the nephridiopore; *tc*, terminal canal; *v*, terminal vesicle; I-III, the three main loops.

(× cir. 45)



TEXT-FIGS. 5-7.

The nephridia of several pre-ctellar segments of *Pontoscolex corethrurus*.

Fig. 5.—A nephridium of the 14th segment.

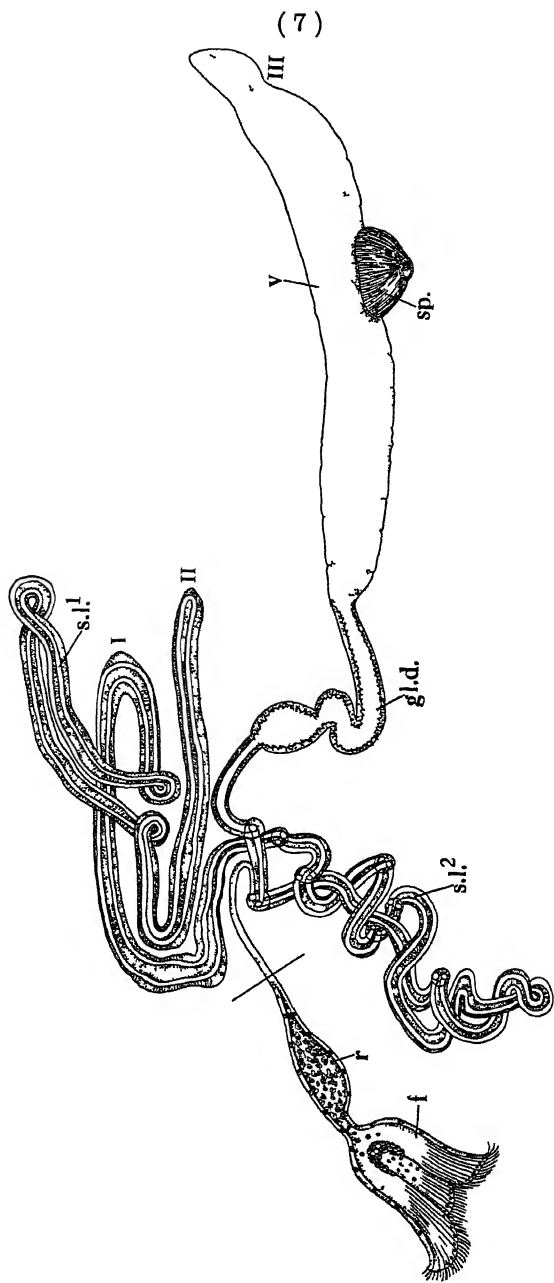
Fig. 6.—A nephridium of the 13th segment.

Fig. 7.—A nephridium of the 3rd segment. In Fig. 7 the funnel is magnified much more than the rest of the nephridium. In Fig. 7 A the same funnel is shown still more magnified. *sl*¹–*sl*⁶, first to sixth subsidiary loops, together forming meronephridial tufts; *tc*, common terminal canal; other letters as in Figs. 2–4. (\times cir. 45.)

clitellar nephridia, but is very well developed in the clitellar nephridia (Text-fig. 3 A) and reminds one of the 'receptacle' behind the funnel of the nephridia of leeches, a fact which was noted by Beddard (5). The funnel is followed by a long narrow neck which pierces the septum behind to enter the body of the nephridium in the succeeding segment. The body of the nephridium consists of three main loops (numbered I, II, and III in Text-figs. 1, 2, and 3) exactly as in that of *Lumbricus*, but it should be noted that loops I and III present differences. The short loop no. 1 consists of two limbs as in *Lumbricus*, but the posterior limb gives off during its course an additional subsidiary loop (*sl*¹) or branch (Eisen's 'spur'), which is absent in *Lumbricus*. As we shall see presently, the formation of this subsidiary loop is the first stage in the branching and division of a holonephridium into several meronephridia. Loop no. III consists of a long and coiled terminal canal of the nephridium, followed by a short but thick specially glandular duct (absent in *Lumbricus*), which opens into a large thin-walled vesicle or bladder. The vesicle forms a very prominent feature of the nephridium and is, in fact, larger in size than the rest of the nephridium; it opens to the exterior through a very prominent terminal sphincter around the nephridiopore. It may be noted that the vesicle extends dorsally much beyond the nephridiopore.

The course of the canal in the nephridial loops of a clitellar nephridium is shown in Text-fig. 8. It is worth noting that the course of the canal is simpler than that in a nephridium of *Lumbricus*, there being only two parallel canals in each of the two limbs of loop no. I and in loop no. II, as compared with three and four in the corresponding limbs and loop in *Lumbricus*. Except for the ciliation of the margin of the funnel, I have not been able to find any ciliated tracts during the course of the canal.

Neither Perrier nor Eisen nor even Beddard realized the essential difference of a subsidiary loop between the nephridium of *Pontoscolex* and that of *Lumbricus*, nor could they follow the course of the canal. Eisen's new terminology for the different parts of the nephridium ('anterior and posterior folds,



TEXT-FIG 8.

A nephridium from a clitellar segment showing the course of the intra cellular canal. Letters as in Figs 2-4 (\times cr. 86)

and a spur') is unnecessary, since all the parts can be named in the same way as those of a nephridium of *Lumbricus*. The neck of the funnel does not enter the 'glandular pouch' as described and sketched by Eisen (7), but enters loop no. I as in the nephridium of *Lumbricus*.

3. THE HOLONEPHRIDIA OF THE CLITELLAR REGION.

The nephridia of the clitellar region (15th or 16th to 23rd or 24th segment) are, in the main, similar to those of the post-clitellar region, but they develop a second subsidiary loop or branch in addition to the one seen in the nephridia of the post-clitellar region, so that there are two subsidiary loops in the clitellar nephridia (Text-fig. 3). The additional secondary loop, characteristic of the clitellar nephridia, is interposed at the beginning of the main loop no. I where the neck of the funnel enters and the terminal canal makes its exit, so that now the neck of the funnel enters this second subsidiary loop and the terminal canal comes out of it. It will be seen that the terminal canal (Text-fig. 2) in the post-clitellar nephridia is long and coiled, while it is short in the clitellar nephridia (Text-fig. 3); apparently a part of it has been incorporated into this second subsidiary loop. The funnel becomes larger and is tri-lobed (Text-fig. 3 A), and the special chamber or 'receptacle' behind the funnel becomes very prominent. The receptacle is filled with coelomic corpuscles in all stages of degeneration, and, in some preparations, is so densely filled as to appear dark and opaque (Text-fig. 3 A). As will be seen from Text-fig. 3, the greater part of the nephridium proper is now formed by the two subsidiary loops or branches. The formation of the second subsidiary loop in the clitellar nephridia is the second stage in the branching and division of a holonephridium into meronephridia.

4. THE MERONEPHRIDIAL TUFTS OF THE PRE-CLITELLAR SEGMENTS.

The nephridia of the pre-clitellar segments (2nd to 15th) seem at first sight to be very different from those of the clitellar and post-clitellar segments, but an examination of the nephridia of

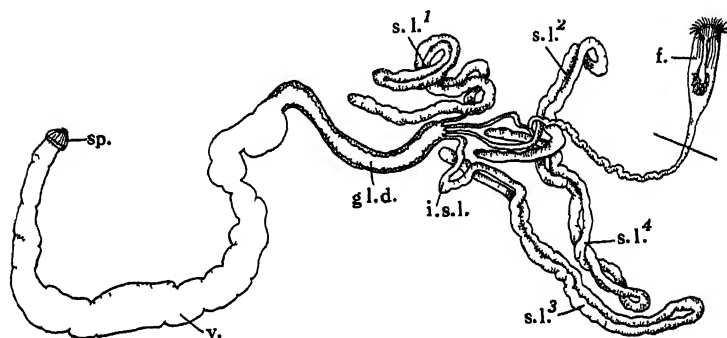
successive segments of this region, from behind forwards, reveals that they have been derived from the clitellar nephridia by a simple process of further branching and division. The funnel is always present and retains its original pre-septal position; similarly, the sphinctered nephridiopores remain faithful to their own segments and retain their original position on the body-wall, although the vesicle is lengthened out to form a thin-walled duct which in the anterior nine nephridia traverses a long distance over several segments and becomes several millimetres in length (Text-fig. 10). It is, therefore, easy to assign the nephridia to their respective segments in spite of their displacement on account of the backward shifting of the inner ends of the septa in these anterior segments.

The first change that takes place in the pre-clitellar nephridia as compared with the clitellar ones is a reduction in size of the main loops nos. I and II, so that the subsidiary loops look much larger in comparison. In the nephridium of the 15th (last pre-clitellar) segment (Text-fig. 4), main loop no. I has become very short and is practically incorporated into the first subsidiary loop, so that the nephridium consists only of the two subsidiary loops and the main loops nos. II and III. In fact, the two subsidiary loops have become so large as to make up the greater part of the nephridium proper.

The next change that takes place is illustrated by the nephridium of the 14th segment (Text-fig. 5); it involves the complete incorporation of main loops nos. I and II into the first subsidiary loop, and the division or break in the continuity of the two subsidiary loops, which are continuous in the nephridia of the 15th segment. Thus the two subsidiary loops in the nephridia of the 14th segment become independent of each other, and, in fact, form two meronephridia, one of which possesses the funnel and the other is without it; their terminal canals are also separate, although both discharge into the common terminal canal which passes into the glandular duct and thence into the vesicle. The disconnected end of the second subsidiary loop now forms the end of a short straight lobe, while that of the first subsidiary loop forms its terminal canal which opens directly into the common terminal canal (Text-fig. 5).

These changes can be easily understood by comparing Text-figs. 4 and 5.

The next change is illustrated in the nephridia of the 13th segment (Text-fig. 6) in which further branching takes place and a third meronephridium is added to the two meronephridia of the 14th segment. The nephridium of the 13th segment, there-



TEXT-FIG. 9.

A nephridium of the 10th segment showing the formation of an incipient subsidiary loop. *sl*¹, incipient subsidiary loop; other letters as in Figs. 2-4. (\times cir. 50.)

fore, consists of three meronephridia, one with a funnel and the other two without funnels, but all of them opening by their separate terminal canals into the common terminal canal. The third meronephridium is formed by a branching of one of the two existing meronephridia. In Text-fig. 9 is shown a nephridium of the 10th segment in which one of the meronephridia is actually seen giving off a branch to form a new incipient meronephridium. It may be noted that the new (third) meronephridium (*sl*)³ is the smallest of the three, as would be expected in the process of successive branching.

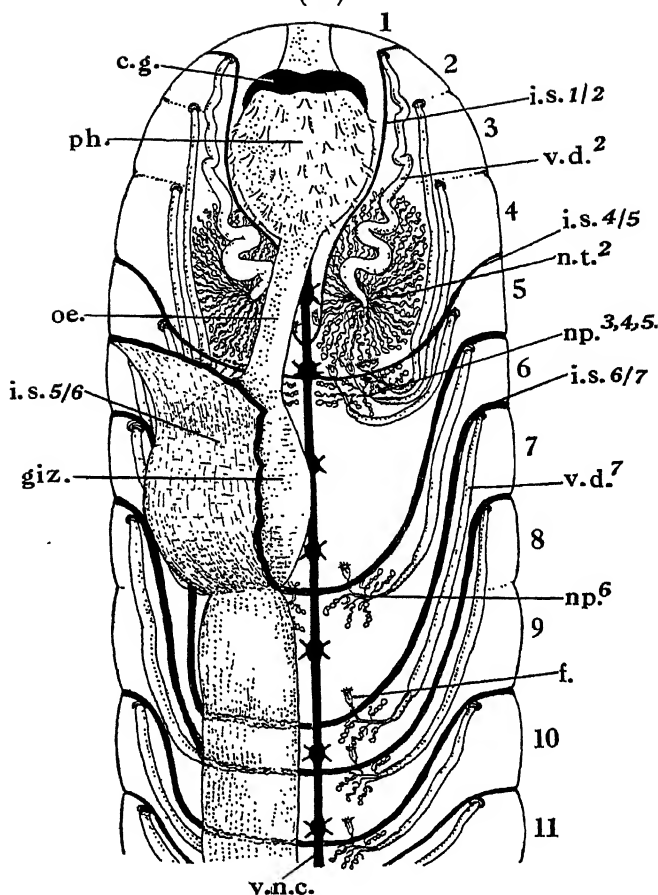
This process of branching continues further so that in the 3rd segment (Text-fig. 7) there are as many as six meronephridia which open by their separate terminal canals into the glandular duct and vesicle.

The extreme degree of branching is reached in the tufted

nephridia of the second segment (Text-fig. 10), where each tuft consists of 80 to 100 small biramous meronephridia, each consisting of a short straight limb and a very long twisted loop, closely resembling the pharyngeal meronephridia of *Pheretima* (1) or *Lampito* (3). The terminal canals of the individual nephridia join and open into a very large and coiled glandular duct leading into the elongated vesicular duct, which in turn opens to the exterior at the anterior end of the second segment (Text-fig. 10).

These nephridial tufts of the 2nd segment (Perrier's *glande à mucosité*) have greatly attracted the attention of previous workers, and I am, therefore, giving a few details about them. They form two distinct tufts, one on each side of the oesophagus, behind the pharynx and in front of the gizzard. These tufts are bounded behind by a thin but distinct septum $4/5$ which is well-developed on the ventral side and is thickly covered with blood-capillaries. Similarly, there is a very thin septum $1/2$ bounding the inner surface of these nephridia and lying between them and the oesophagus. Both these septa were unfortunately missed by Stephenson (9). Three facts about these nephridia need to be emphasized. The first is that each tuft possesses only one funnel which is pre-septal, and lies between the oesophagus and the septum $1/2$, i.e., on the inner or anterior face of this septum. Beddard (5) was mistaken in his observation that these nephridia had three funnels on each side. It is true that three funnels lie just behind these tufted nephridia, as noted by Beddard, but they belong to the next three tufts of nephridia belonging to the 3rd, 4th, and 5th segments, as shown in Text-fig. 10, and not to the tufted nephridia of the second segment. The second point to be emphasized is that the vesicular duct of each of these nephridial tufts opens to the outside on segment ii as 'observed' by Perrier (8) and Beddard¹ (5) and not into the

¹ Beddard erroneously states that it opens on the first segment; his diagram shows it as opening on the fourth segment. Since the vesicular ducts of the nephridia of the 2nd, 3rd, 4th, and 5th segments open similarly to the exterior on successive segments. Beddard apparently mistook the duct of the nephridium of the 4th segment for that of the nephridium of the second segment.



TEXT-FIG. 10.

The general plan of the nephridial system in the first ten segments (semi-diagrammatic). *cg*, cerebral ganglia; *f*, pre-septal funnel of the nephridium of the 7th segment; *giz*, gizzard; *is* 1/2, *is* 4/5, *is* 5/6, *is* 6/7, intersegmental septa separating the several numbered segments; the septum 5/6 covering the gizzard is cut to expose a part of the gizzard and the nephridia of the 3rd, 4th, and 5th segments on the right side; *np*^{3, 4, 5}, tufted nephridia of the 3rd, 4th, and 5th segments with their respective pre-septal funnels in front, and their vesicular ducts leading to and opening on their respective segments; *np*⁶, tufted nephridium of the 6th segment; *nt*², nephridial tuft of the 2nd segment with its pre-septal funnel between septum 1/2 and the ventral nerve-cord; *oe*, oesophagus; *ph*, pharynx; *vd*², *vd*⁷, vesicular ducts of the nephridial tufts of the 2nd and 7th segments. (× cir. 7.)

pharynx as erroneously 'described' by Stephenson (9). I have followed the course of the vesicular duct both in dissections and in transverse sections, and have verified that it opens on the body-wall through a sphinctered aperture. It may be noted here that as the vesicles of the anterior nine pairs of nephridia are lengthened out to form ducts traversing a long distance before opening to the exterior, their sphincters come to lie at their terminal ends or, in other words, the vesicles do not extend beyond the sphincters as they do in the clitellar and post-clitellar nephridia. The third and the most important point to be borne in mind is that previous workers did not appreciate the fact that each tuft consists of a large number of small but separate meronephridia, opening into a common vesicular duct. Beddard (5) thought that this large anterior nephridium was formed by the fusion of two or three embryonic nephridia—he did not realize that it consisted of about 80 to 100 small meronephridia. Similarly, Stephenson (9) stated that 'the nephridium of segment ii forms a large and close coil on the side of the oesophagus'. Apparently he saw the large coiled glandular duct and vesicle and not the large number of meronephridia. Even in his monograph (11) he does not refer to these nephridia as tufted nephridia. Like Beddard he also did not realize that he was dealing with a tuft of multiple meronephridia.

The nephridia of the 3rd, 4th, and 5th segments have become telescoped together, and lie in the 5th segment because the septa $2/3$ and $3/4$ are absent. The funnels of these three nephridia, therefore, lie close together on the anterior face of the septum $4/5$, and their bodies also lie close together in segment 5 (Text-fig. 10); but their vesicles are very much prolonged and travel forward to open on their respective segments (3rd, 4th, and 5th), thus proving that they belong to these three segments. Each nephridium is tufted and consists of five or six distinct meronephridia. The nephridia of these three segments do not 'interlace or communicate with one another', as was suspected by Stephenson (9). It was the three funnels of these three nephridia that were thought by Beddard to belong to the nephridia of the second segment.

From the foregoing description of the nephridia of the

pre-clitellar segments (segments 2 to 14) and an examination of Text-figs. 4 to 7, and 9 and 10, it becomes clear that the single holonephridium of the clitellar region has become branched and divided into a number of meronephridia, all of which still open to the exterior through a common glandular duct and vesicle. It could be maintained that each tuft still represents one nephridium, as there is one funnel, one vesicle, and one nephridiopore for each tuft, but at the same time it must be recognized that each constituent of a tuft is morphologically equivalent to a meronephridium of *Pheretima* (1) or *Lampito* (3). One is justified in maintaining that the nephridium in *Pontoscolex* is in the process of dividing up, although the process is not complete as it is in the pharyngeal nephridia of *Lampito* (3) or *Woodwardiella* (4) where each meronephridium opens into the pharynx by its own separate ductule.

5. DISCUSSION AND SUMMARY.

The phylogenetic relationship between the meganephridia and micronephridia has been discussed by several workers. Beddard (6) did not believe that 'the diffuse nephridia (micronephridia) were the outcome of a branching and specialization of the paired nephridia (meganephridia)', but thought that 'both kinds of excretory organs are equally ancient'. Stephenson (11) agrees with Beddard and says, 'we do not even know whether the original single nephridium became divided into a number, or whether instead of a single nephridium on each side several developed independently of each other in each segment. . . . I do not myself think that the tufted nephridia represent a stage in the division of a meganephridium or that the individual components of a tuft represent so many micronephridia, any more than the branches of the alimentary canal of a Polyclad represent so many independent organs.' Unfortunately Stephenson did not have a complete account of the nephridial system of *Pontoscolex* available to him, or else he might have written differently. A comparison of the nephridia of the post-clitellar and clitellar regions with those of the several segments of the pre-clitellar region of this earthworm affords convincing evidence, from the point of view of comparative

anatomy, for the hypothesis that the original single meganephridium (holonephridium) at first branches and then divides into a number of meronephridia. It is this process of branching and division that leads to the formation of tufted nephridia which are found in each of the segments 2 to 13. When we find meganephridia in the posterior segments and the tufted meronephridia in a graded series in the anterior segments, there can be no other explanation except that the posterior nephridia are primitive and least modified, and that the anterior tufted nephridia are derived by a process of branching from the posterior nephridia, particularly when we can follow all the stages of branching and division in the nephridia of the same worm. Further, on comparing the individual components of the tufted nephridia with the septal and integumentary nephridia of *Pheretima* (1), one cannot fail to be struck with their essential similarity and morphological equivalence. Each component of a tuft in *Pontoscolex* is, in fact, a meronephridium, only one of the components retains the original pre-septal funnel, while all others are without funnels, but all of them open into the common terminal canal through their separate slender terminal canals. If we compare the condition in *Pontoscolex* with that of the pharyngeal tufted nephridia of *Lampito* (3) and *Woodwardiella* (4), in which the funnel is lost and all the components of the tuft open separately by their own canals into the pharyngeal lumen, we can have no hesitation in asserting that the tufted nephridia of *Pontoscolex* do represent a stage in the division of a meganephridium. Stephenson's comparison of the tufted nephridia with the branches of the alimentary canal of a Polyclad is unfair and inapplicable, since in no Polyclad do we find the several branches of the gut completely separating off and opening separately to the exterior as we find in the case of the several components of the pharyngeal tufted nephridia of *Lampito* and *Woodwardiella*, each of which opens separately into the pharynx.

In view of the fact that the tufted nephridia of *Pontoscolex* not only retain their large original funnel but also the original glandular duct and vesicle, it is justifiable to hold that each tuft is still a single nephridium, but it must be conceded

that each nephridium is a very much branched and divided meronephridial tuft. I have already demonstrated that the pharyngeal nephridial tufts in *Pheretima* arise during development by a process of budding at the nephridial end of the pharyngeal duct (2).

To summarize, the nephridial system of *Pontoscolex corethrurus* consists of holonephridia in the process of branching in the post-clitellar and clitellar segments, and of very much branched and divided meronephridial tufts in the pre-clitellar segments. Since the pre-clitellar tufts exhibit all stages of branching, and their individual components are morphologically equivalent to the meronephridia of *Pheretima*, *Eutyphoeus*, and *Lampito*, we are justified in holding that the tufted nephridia represent a stage in the division of a holonephridium into meronephridia.

6. APPENDIX: EISEN'S SO-CALLED 'SAFETY VALVES'.

Eisen (7) described the intra-typhlosolar canals of *Pontoscolex* in these words, 'Throughout the length of the typhlosole there exist in the upper part of this organ a great number of internal ciliated canals enclosed by a muscular investment continuous with the circular muscle layer of the intestine . . . these intra-typhlosolar canals originate in the interior of the typhlosole and then bend sufficiently to open into the upper part of the intestine at its junction with the typhlosole . . . these canals probably serve as safety valves, enabling the typhlosole to discharge superfluous blood into the intestine'.

Eisen's description of the situation of these short canals is correct, but he is entirely mistaken in thinking that they are enclosed by a muscular investment and that they are connected with the blood-sinus of the typhlosole. The typhlosole, like the rest of the intestinal epithelium, is ciliated throughout its length and all that these short canals represent are small pockets of this ciliated epithelium at the junction of the typhlosole with the upper part of the intestine. These pockets or canals are lined all along by ciliated epithelium and have no muscular investment, nor are they connected with the blood-sinus of the typhlosole.

Each canal is really an ingrowth of the intestinal epithelium into the typhlosole, communicating with the lumen of the intestine through an elongated slit-like opening, about $72\ \mu$ in length. Each canal is about $144\ \mu$ in length and ends blindly in the base of the typhlosole. The so-called intra-typhlosolar canals of Eisen are, therefore, only small pockets of the intestinal lumen into the typhlosole apparently to increase the ciliated and glandular surface, and have no connexion whatsoever with the blood-vascular system.

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Studies on the Structure, Development, and Physiology of the Nephridia of Oligochaeta.

IV. The Enteronephric System in *Megascolex cochinensis*, with Remarks on Vestigial Nephridia.

By

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With 8 Text-figures.

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1. INTRODUCTION.

I HAVE already described in the pages of this journal the 'enteronephric' type of nephridial system in four genera of earthworms, namely, *Pheretima*, *Lampito*, *Woodwardiella*, and *Tonoscolex* (1, 2, 3, 4, and 4a), and in this memoir I am adding another earthworm, i.e. *Megascolex cochinensis* Stephenson (sub-family *Megascolecinae*), to the category of earthworms possessing this type of nephridial system. In summarizing my observations on the nephridia of *Woodwardiella* (4) in 1926 I remarked that 'the chief point of interest which may well be emphasized here is that the enteronephric type of nephridial system seems to be widely spread amongst earthworms, different families of which would seem to need re-examination from the point of view of the occurrence and origin of the "enteronephric" excretory system'.

My prediction has proved correct, as since 1926 I have been able to find this type of nephridial system in two other genera (*Tonoscolex* and *Megascolex*).

Megascolex is a large genus comprising as many as 117 species, of which 52 are found in India, Burma, and Ceylon. The genus is defined as micronephridial, although Stephenson (10) states that 'there are many varieties of nephridial arrangements in the genus *Megascolex*'. The study of the nephridia in this genus has suffered because they are so minute as to need properly fixed material for microscopic examination, and such material has not been easily available. Moreover, a proper examination of the ducts and openings of the nephridia cannot be made without section-cutting, which is not often resorted to by systematists. Further, as I have emphasized in part I of this memoir, it is necessary to examine the nephridia of all the regions of an earthworm to get a complete picture of the nephridial system. In *Megascolex cochiniensis* the enteronephric system occurs from the 74th segment to the posterior end of the worm, so that even a careful observer like Stephenson, who probably did not dissect the worm behind the 74th segment, missed the most important fact about its nephridial system. I believe that a careful study will reveal that many, if not all, of the species of *Megascolex* possess an enteronephric type of nephridial system, essentially similar to that of *Pheretima* (1).

The species *Megascolex cochiniensis* was instituted by Stephenson (9), who described its excretory system correctly as micronephridial, 'the nephridia occurring as bushy tufts in the pre-clitellar and clitellar regions, and in a transverse row (not a single line of nephridia) in the post-clitellar region'. Later, in his monograph on the *Oligochaeta* (11), Stephenson records that the paired segmental tufts (of nephridia) in the anterior part of the body discharged externally; I have now found that the anteriormost pair of these tufts discharges into the pharynx and not externally, another important fact missed by Stephenson.

I am very grateful to Miss Mary Chandy of the Isabella Thoburn College, Lucknow, who kindly brought me well fixed specimens of this earthworm from Kottayam (Travancore),

South India. My best thanks are also due to Dr. G. E. Gates of Rangoon (Burma) who kindly sent me specimens of *Woodwardiella pumila*.

2. THE NEPHRIDIAL SYSTEM OF MEGASCOLEX COCHINENSIS.

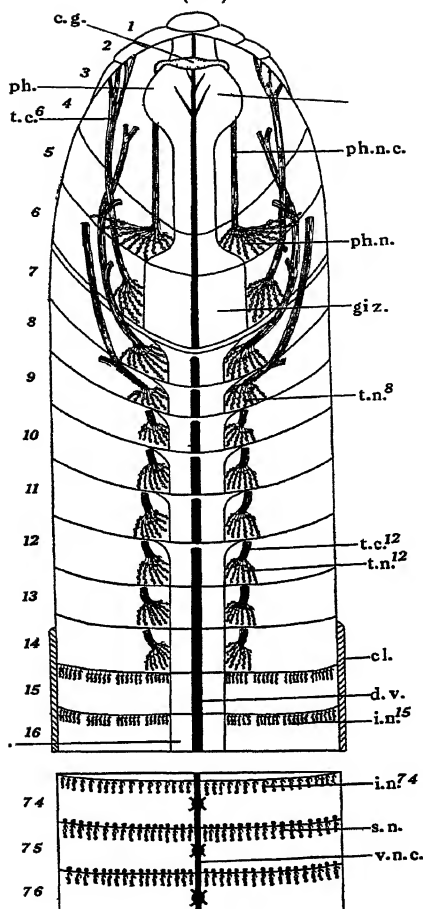
(a) The General Plan of the System.

The general plan of the nephridial system of this earthworm (Text-fig. 1) is similar to that of *Pheretima* (1), but there are important differences. The nephridia are all minute and are beyond doubt meronephridia. They can be classified into four categories: (1) the septal meronephridia which are found in the posterior 101 to 150 segments behind the 74th segment, the total number of segments in the worm being 175 to 224; these nephridia possess funnels and are enteronephric, discharging their excretory products into the intestine in each segment; (2) the integumentary meronephridia which occur in three clitellar segments¹ behind the 14th, and behind the clitellum right up to the 74th segment; these nephridia have no funnels and are exonephric; (3) the tufted meronephridia which are found in the 6th to the 14th segment; they are also without funnels and are exonephric; (4) the pharyngeal meronephridia which form a pair of large tufts in the 5th segment; they also have no funnels, but discharge their excretory products into the lumen of the pharynx, and are, therefore, enteronephric. These four kinds of nephridia are represented semi-diagrammatically in Text-fig. 1.

(b) The Enteronephric Septal Meronephridia, and their Ducts and Openings.

The septa of the first 74 segments do not bear any nephridia, the first septum having nephridia attached to it being the septum 74/75. From this septum backwards all the septa bear nephridia, so that the septal nephridia occur in all the posterior 101 to 150 segments. Each septum bears 35 to 40 nephridia on each of its right and left sides, attached to the outer border of

¹ The clitellum extends from the 14th to the 17th segment, both inclusive.



TEXT-FIG. 1.

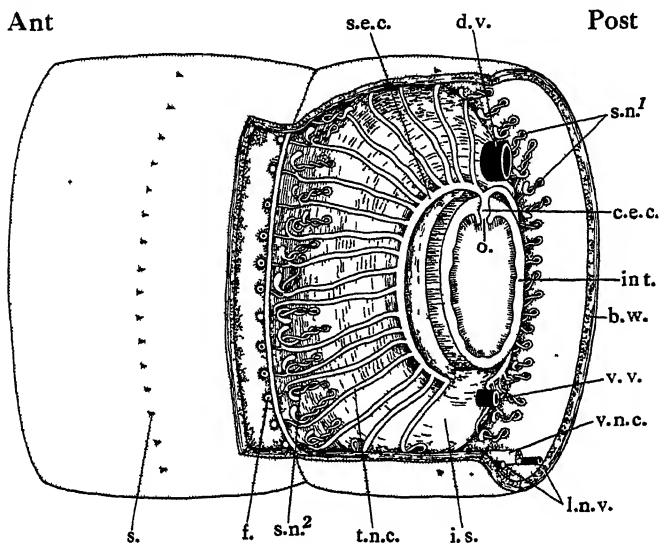
The general plan of the nephridial system in *Megasclex cochi-nensis* (diagrammatic). Numerals on the left indicate the serial numbers of segments. For clearness fewer nephridia are shown than are actually present in each segment. *cg*, cerebral ganglia; *cl*, clitellum; *dv*, dorsal vessel; *giz*, gizzard; *in*¹⁵, integumentary nephridia in groups in the 15th segment; *in*⁷⁴, integumentary nephridia in a row in the 74th segment; *int*, intestine; *o*, opening of the pharyngeal nephridial duct into the lumen of the pharynx; *ph*, pharynx; *phn*, pharyngeal nephridia; *phnc*, bundle of canals of the pharyngeal nephridia; *sn*, septal nephridia; *tc*⁶, *tc*¹², bundles of terminal canals of the tufted nephridia of the 6th and 12th segments; *tn*⁸, *tn*¹², tufted nephridia of the 8th and 12th segments; *nc* ventral nerve-cord.

each intersegmental septum, at the place where the septum joins the body-wall. The nephridial funnels form a linear row on the anterior face of each septum and the bodies of the nephridia form a row on its posterior face (Text-fig. 1). In structure each nephridium is identical with a septal nephridium of *Pheretima*, consisting of a short straight limb and a long twisted loop (Text-fig. 8), but the terminal canal is very long and runs across the whole width of the septum to enter the septal excretory canal, which runs close to the inner border of each septum, where it is inserted on the intestine at each intersegmental place. The posterior face of each septum, therefore, bears a large number of terminal canals on its surface, as many as the number of nephridia, and the two septal excretory canals, one on each side (Text-fig. 2). In size the septal nephridia vary; most of them are large in size, but there are a few which are smaller than the rest (Text-fig. 8); it is possible that new septal nephridia keep on developing throughout life. I shall discuss this point in the appendix in connexion with the vestigial nephridia of this worm.

The two septal excretory canals of each septum meet in the mid-dorsal line just on the outer surface of the intestine and form a common excretory canal which immediately penetrates the shallow typhlosole, and opens into the lumen of the gut in the mid-dorsal line. The arrangement of the nephridia and their funnels, the position of their terminal canals and septal excretory canals, and the manner of opening of the common excretory canal through the typhlosole into the lumen of the intestine are illustrated in Text-figs. 2 and 3.

Since the septal nephridia resemble those of *Pheretima* not only in their structure and size, but also in their enteronephric character, it is not necessary to give a detailed description of the entire system, and I am, therefore, only enumerating the points wherein the septal enteronephridial system of *Megascolex cochinensis* differs from that of *Pheretima*. (1) In *Pheretima* the septal nephridia begin from the septum 15/16 and extend throughout the rest of the body, but in *Megascolex cochinensis* they begin far behind, i.e. from septum 74/75, the first 74 segments being without septal nephri-

dia. (2) In *Pheretima* the septal nephridia coexist throughout their extent with the integumentary nephridia, but in *Megascolex cochiniensis* there are no integumentary

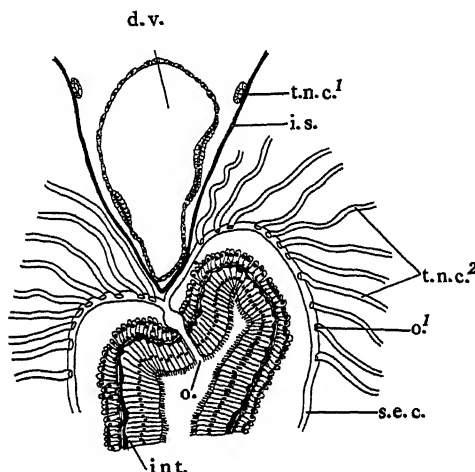


TEXT-FIG. 2.

A diagrammatic representation of the enteronephric nephridial system and its relative position as found behind the 74th segment in *Megascolex cochiniensis*. *bw*, body-wall; *cec*, common excretory canal; *dv*, dorsal vessel; *f*, funnel of a septal nephridium; *int*, intestine; *is*, intersegmental septum; *lnv*, lateral neural vessels; *o*, opening of the common excretory canal into the lumen of the intestine; *s*, seta; *sec*, septal excretory canal; *sn¹*, *sn²*, septal nephridia of the right and left sides; *vnc*, ventral nerve-cord; *vv*, ventral vessel; *tnc*, terminal nephridial canal.

nephridia in the last 101 to 150 segments in which septal nephridia alone occur. (3) In *Pheretima* these nephridia are present on both the anterior and posterior faces of each septum, thus forming four rows of nephridia in each segment, but in *Megascolex cochiniensis* they are present only on the posterior face of each septum, with, of course, pre-septal funnels on the anterior face, thus forming only two rows in each segment, one on each side. (4) The septal nephridia of *Pheretima*

lie wholly in one segment, both the funnel and the body of the nephridium lying together in the same coelomic chamber, but in *Megascolex cochinensis* the funnel always lies in the



TEXT-FIG. 3.

A reconstruction of three consecutive transverse sections through the intestinal region (behind the 74th segment) of *Megascolex cochinensis* showing the two septal excretory canals uniting and opening into the lumen of the intestine; *dv*, dorsal vessel; *int*, wall of the intestine; *is*, intersegmental septum; *o*, opening of the common excretory canal into the intestinal lumen; *o¹*, opening of a terminal nephridial canal into the septal excretory canal; *sec*, septal excretory canal; *tnc¹*, terminal nephridial canal cut transversely; *tnc²*, terminal nephridial canals cut longitudinally. (\times cir. 66.)

segment preceding the one containing the body of the nephridium. (5) In *Pheretima* the septal excretory canals run along the outer border of the septum, while in *Megascolex cochinensis* they run along its inner border. (6) As a consequence of the difference in the position of the septal excretory canals, the terminal excretory canals in *Megascolex cochinensis* have to travel across the whole width of the septum to reach their septal excretory canal and are therefore very long, while the terminal canals in *Pheretima* are much shorter.

(7) In *Pheretima* the septal excretory canals discharge into two supra-intestinal excretory ducts, which run all along the length of the intestine, and open into the intestinal lumen in each segment, either to the right or to the left of the typhlosole; thus the septal nephridial system is continuous from segment to segment and forms one connected system throughout the intestinal region of the body; but in *Megascolex cochinesis* there is no supra-intestinal excretory duct at all, and the septal excretory canals of each septum unite in the mid-dorsal line to form one common excretory canal which opens immediately into the lumen of the intestine in each segment. There is thus no continuity of the septal nephridial system from segment to segment, as we find in *Pheretima*, the septal nephridial system of each segment in *Megascolex cochinesis* being independent.

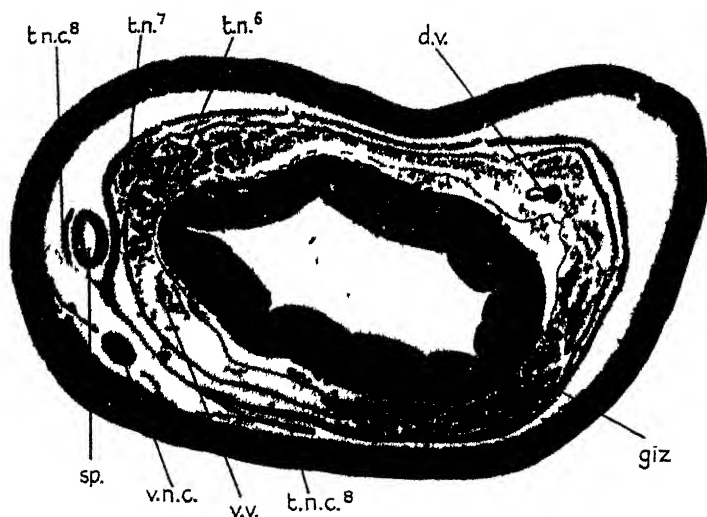
(c) The Integumentary Meronephridia.

The integumentary meronephridia (Text-fig. 1) occur from the 15th to the 74th segment. In three of the clitellar segments (15th to 17th) the nephridia are very numerous and are so thickly crowded as to form a number of closely set groups on the parietes in the anterior part of each segment, but each nephridium of a group is discrete and opens on the body-wall by its own short terminal duct. In the post-clitellar segments, on the other hand, the nephridia are fewer and evenly distributed and form a narrow band in the anterior part of each segment just behind the intersegmental septum. They are disposed exactly in the same manner as the septal nephridia in the posterior segments. These nephridia resemble the septal nephridia in structure, but they lack a funnel and their short terminal canals open on the surface of the body-wall.

(d) The Tufted Meronephridia.

The tufted nephridia (Text-figs. 1 and 4) occur in nine segments, i.e. from the 6th to the 14th segment. In each of these nine segments there is a pair of compact tufts of nephridia which lie in the posterior part of the coelomic chamber, and are closely connected with the posterior septum of the segment through

connective tissue strands. Each tuft lies close to the gut and the ventral nerve-cord and forms a bushy structure consisting of 40 to 50 minute nephridia in one bunch. Unlike the integumentary nephridia which extend over the parietes from the ventral



TEXT-FIG. 4.

A microphotograph of a transverse section of *Megasculex cochinensis* through the region of the gizzard, showing the tufted nephridia of the 6th and 7th segments, and the bundle of canals of the tufted nephridia of the 8th segment. *dv*, dorsal vessel; *giz*, gizzard; *sp*, spermatheca; *tn*⁶, *tn*⁷, tufted nephridia of the 6th and 7th segments; *tnc*⁸, bundle of canals of the tufted nephridia of the 8th segment; *vnc*, ventral nerve-cord; *vv*, ventral vessel. (× cir. 30.)

nerve-cord to the dorsal vessel, the tufted nephridia float in the coelomic cavity away from the parietes. The terminal canals of the nephridia of each tuft are united together by connective tissue to form a bundle of ductules which, in a dissection, looks like a flat band running forward on the inner surface of the body-wall; on its way the bundle breaks up and the ductules open individually on the body-wall through their several minute

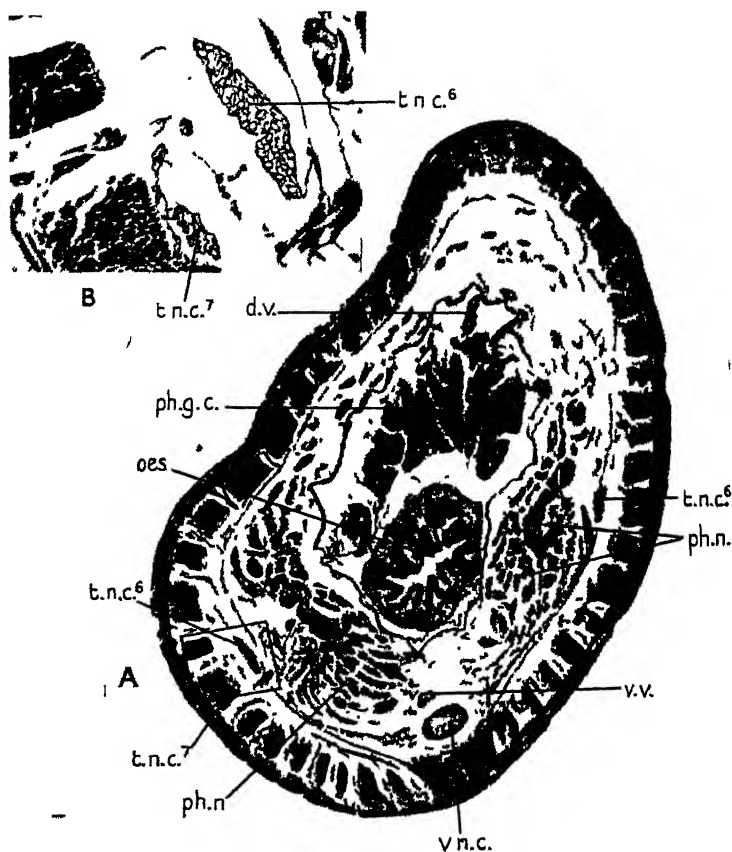
apertures. In transverse sections the bundles of ductules present a honeycombed appearance (Text-figs. 4 and 5 B). It is noteworthy that the ductules of these tufted nephridia retain their individuality all along their course which may extend over three, four, or five segments in the case of the anterior pairs of tufts (of the 6th, 7th, and 8th segments), so that the ductules open several segments in front of the one to which they belong. Thus the ductules of the nephridia of the 6th segment open on the body-wall of the 2nd and 3rd segments, and those of the 7th segment open on the 4th, 5th, and 6th segments (Text-fig. 1). The bundles of ductules of the tufted nephridia of segments 9 to 14 open on the body-wall at the anterior ends of their own segments, close to the septum in front.

These nephridia resemble the integumentary nephridia in their structure and also in their exonephric character.

(e) The Pharyngeal Meronephridia.

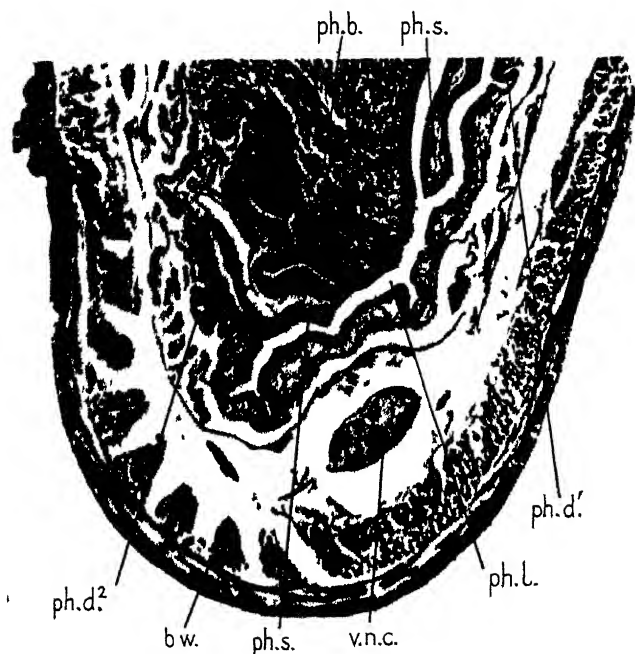
The pharyngeal nephridia form a single pair of large tufts in the fifth segment, behind the pharynx and just in front of the gizzard (Text-figs. 1 and 5). These tufts contain more numerous nephridia (80 to 100 on each side) than are present in the tufts of the succeeding nine segments; they are, therefore, much larger in size and occupy the greater part of the coelomic chamber of the 5th segment. In size and structure each of these nephridia resembles a component of the tufted nephridia, consisting of a short straight lobe and a long twisted loop, without a funnel. The terminal canals of these nephridia also run close together and form a band-shaped bundle on each side, consisting of separate canals held together by connective tissue, like the bundles of ductules of the tufted nephridia. Each band of discrete canals runs forward along the ventro-lateral aspect of the pharynx, but about the middle of the 4th segment the canals unite together to form a large common duct which runs for a very short distance and opens through the floor of the pharynx into its lumen, on each side of the mid-ventral line in the anterior part of the 4th segment (Text-fig. 6). The pharyngeal nephridia are, therefore, enteronephric.

It will be readily seen that the tufts of pharyngeal nephridia



TEXT-FIG. 5.

A. A microphotograph of a transverse section of *Megascolex cochinensis* passing through the pharyngeal nephridial tufts of the 5th segment and the bundles of canals of the tufted nephridia of the 6th and 7th segments (\times cir. 27). B. Part of Text-fig. 5 A (marked by a rectangle) highly magnified to show the bundles of terminal canals of the tufted nephridia of the 6th and 7th segments (\times cir. 116). *dv*, dorsal vessel; *oes*, oesophagus; *phgc*, pharyngeal gland cells; *phn*, pharyngeal nephridia; *tnc*⁶, bundle of terminal canals of the tufted nephridia of the sixth segment; *tnc*⁷, bundle of terminal canals of the tufted nephridia of the 7th segment; *vnc*, ventral nerve-cord; *vv*, ventral vessel.



TEXT-FIG. 6.

A microphotograph of the ventral part of a transverse section of *Megascycolx cochinensis* through the pharyngeal region (anterior end of the 4th segment) showing the pharyngeal nephridial duct of the right side opening into the lumen of the pharynx and that of the left side lying close to the pharyngeal wall (it opens a few sections in front). *bw*, body wall; *phb*, pharyngeal bulb; *phd*¹, duct of the pharyngeal nephridia of the right side opening into the lumen of the pharynx; *phd*², duct of the pharyngeal nephridia of the left side meeting the wall of the pharynx to open a few sections in front; *phl*, pharyngeal lumen; *phs*, pharyngeal shelf; *vnc*, ventral nerve-cord. (\times cir. 55.)

are serially homologous with the succeeding nine pairs of tufted nephridia, but unlike the latter, they open into the pharynx, and not to the exterior. It was this opening into the pharynx which was missed by Stephenson (9).

3. DISCUSSION AND SUMMARY.

In summarizing and discussing my observations on the nephridial system of *Tonoscolex* (4a) I remarked that 'an intensive study of the nephridial systems of other Megascolecine earthworms will probably reveal other intermediate stages which may enable us to bridge the gap between exonephric and enteronephric conditions of the nephridial system'. One such intermediate stage is now provided by the nephridial system of *Megascolex cochinensis*, as far as the origin of the pharyngeal and tufted nephridia is concerned.

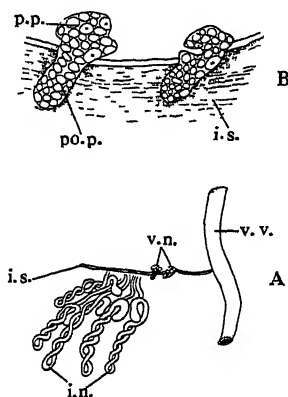
In Part III of this series of memoirs we have seen how the meronephridial tufts of the pre-clitellar region of *Pontoscolex* arise from the holonephridia of the posterior segments of the same worm by a process of branching and division. The tufted meronephridia of *Megascolex cochinensis* can be easily derived from those of *Pontoscolex* by the disappearance of the pre-septal funnel at one end, and the glandular duct and the terminal vesicle on the other, so that the terminal canals of all the several meronephridia remain discrete and open directly and independently on the body-wall. That the tufted nephridia of *Megascolex cochinensis* are so derived from those of *Pontoscolex* is supported by the fact that the bands of terminal canals of the tufted nephridia of *Megascolex cochinensis* still travel forward over several segments and open far in front of their own segments just as the terminal vesicles of the meronephridial tufts do in *Pontoscolex*. Further, on comparing the tufted nephridia of the 6th to the 14th segments in *Megascolex cochinensis* with its pharyngeal nephridia of the 5th segment, we realize that both these types of nephridia are serially homologous and belong to the same category, the only difference being that the pharyngeal tufts open into the lumen of the pharynx, while the following nine pairs of tufts still open to the exterior on the body-wall. We are, therefore, justified in concluding that in *Megascolex cochinensis* we have the beginnings of the enteronephric process whereby the bundles of terminal canals of the anterior-most pair of tufts of nephridia have changed over their place of

opening from the outer body-wall into the pharyngeal lumen. In *Pheretima* this process has proceeded farther, since in that worm three pairs of tufts open into the pharynx and buccal cavity, but in *Megascolex cochinensis* the process has just begun since it is only the anteriormost pair that opens into the pharynx, while the succeeding nine pairs of tufts still open to the exterior. It is evident, therefore, that the pharyngeal nephridia have originated from the tufted nephridia by a change in their place of opening. What physiological need led to this change in position of the place of opening? It may be the need for conservation of water in the excretory fluid, but we shall discuss this point in a subsequent part of this memoir.

To summarize: the nephridial system of *Megascolex cochinensis* consists only of meronephridia, which are arranged in four sets: the septal, the integumentary, the tufted, and the pharyngeal, the nephridia of only one set being present in any one segment. Of these, the septal nephridia occur in the posterior 101 to 150 segments of the body behind the 74th segment; these nephridia have pre-septal funnels and discharge their excretory products into the intestine in each segment through a pair of septal excretory canals which meet to form a common excretory canal. The nephridial system of each segment is separate and independent of that of the preceding and succeeding segments. These septal nephridia are enteronephric. The integumentary nephridia occur in segments 15 to 74, while the tufted nephridia occur in segments 6 to 14; the nephridia of both these sets are exonephric. The pharyngeal nephridia form a single pair of tufts in the 5th segment and open into the pharyngeal lumen; these nephridia are, therefore, enteronephric. Except the septal nephridia, all the other nephridia are without funnels.

Postscript.—Since the completion of the manuscript of this paper I have come across another species of *Megascolex*, i.e. *Megascolex konkanensis*, in which the nephridial system is arranged almost identically on the same lines as in *Megascolex cochinensis*, the only difference being that the septal nephridia begin from the 100th segment instead of from the 74th. The integumentary nephridia extend from the

15th to the 99th segment. The tufted and the pharyngeal nephridia have the same position as in *Megascolex cochinensis*. This earthworm is extremely long, a specimen 415 mm. in length having as many as 370 segments; in view of the great length of the worm it is not surprising that the septal nephridia



TEXT-FIG. 7.

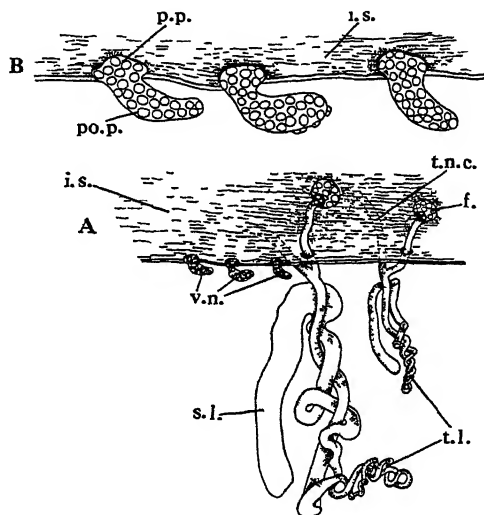
- A. Five adult integumentary and two vestigial septal nephridia in the body region of *Woodwardiella pumila* (\times cir. 63).
 B. The septal vestigial nephridia, highly magnified (\times cir. 214).
in, cluster of five integumentary nephridia; *is*, intersegmental septum; *pp*, pre-septal (funnel) part of a vestigial nephridium; *pop*, post-septal (body) part of the same; *vn*, vestigial nephridia; *vv*, ventral vessel.

have been pushed farther back and occur in the posterior 270 segments.

4. APPENDIX: VESTIGIAL NEPHRIDIA.

In Part II of this memoir I have described examples of vestigial funnels of nephridia in *Hoplochaetella* and *Lampito*. I am now describing cases of vestigial nephridia in *Woodwardiella pumila* and *Megascolex cochinensis*. As far as I know there is no record in literature of vestigial nephridia in adult earthworms. Specimens of these two worms were fully clitellate and had their external genital markings and internal genital apparatus completely developed.

In *Woodwardiella pumila* each segment of the body region has a cluster of four to five minute meronephridia, on each side, just behind each intersegmental septum. These nephridia are attached to the body-wall, and lie so close together as to give the appearance of a single nephridium in dissection. They are undoubtedly integumentary nephridia without funnels.



TEXT-FIG. 8.

- A. Two fully formed and three vestigial septal nephridia in the posterior region of the body of *Megascolex cochinensis* (\times cir. 70). B The vestigial septal nephridia, highly magnified (\times cir. 235). *f*, funnel; *is*, intersegmental septum; *pp*, pre-septal (funnel) part of the vestigial nephridium; *pop*, post-septal (body) part of the same; *sl*, straight lobe; *tl*, twisted loop; *tnc*, terminal nephridial canal on the posterior face of the septum; *vn*, vestigial nephridia.

But an examination of a stained mount of a septum reveals the presence of two or three vestigial nephridia on each septum, which look exactly like embryonic nephridia. A reference to Text-fig. 7 will show that these vestigial nephridia are really septal nephridia with funnels, which have become arrested in

development. They show clearly a pre-septal funnel-rudiment and a post-septal body-rudiment of the nephridium.

It is remarkable that in this earthworm the only functional nephridia are integumentary; there are no functional septal nephridia and yet the vestiges of these are present on the septa.

Similar vestigial nephridia are met with in *Megascolex cochinensis*. Scattered here and there between well developed functional septal meronephridia are found a number of vestigial septal nephridia showing rudiments of a pre-septal funnel and a post-septal body. They also look like embryonic nephridia arrested in development. The functional septal nephridia in this worm are of different sizes; some are large and others small (Text-fig. 8), the smaller ones being only half the size of the larger ones. It is possible that the smaller nephridia are young nephridia in the course of development; if it is so, the 'vestigial' nephridia may really be rudimentary nephridia which develop later into fully formed septal nephridia. But as there is no evidence yet available that they do so develop, it is best to regard them as vestigial nephridia of the same kind as those of *Woodwardiella pumila*.

The 'Dorsal Organ' of the Embryo of *Campodea*.

By

O. W. Tiegs

(Department of Zoology, University of Melbourne.)

With Plate 1, and 3 Text-figures.

IN his well-known work on the embryology of apterygote insects, written over forty years ago, Uzel recorded the presence of a 'dorsal organ' in the embryo of *Campodea staphylinus*. It was described as a circular thickening of the provisional body-wall, located in a median dorsal position immediately behind the developing head, but devoid of any very distinctive structural features. Like the problematical 'dorsal organ' (pre-cephalic organ) of collembolan embryos it was found to be a purely embryonic structure, and disappeared without a vestige before the larva left the egg. Its function was unknown.

The purpose of the present work has been to determine whether the 'dorsal organ' of *Campodea* is similar to the remarkable structure of the same name of symphyliid and collembolan embryos (Tiegs, 1940 and 1942).

My observations have been made on *Campodea fragilis*, a species which has been introduced from Europe into Australia, where it is now widely spread, and is not uncommonly met with under decaying vegetation in suburban gardens (Womersley, 1939). The adults lay freely in captivity. The eggs are placed, in small clumps, within fragments of rotting vegetation. They are spherical, and measure usually about 0.4 mm. in diameter. The newly laid egg is white, with a soft chorion, but the latter soon hardens into a brown horny shell and is then very impermeable to fixatives.

In order to fix the embryo the chorion must first be punctured. In practice I have found it best to immerse the eggs in water, puncturing the chorion with a very finely ground needle

under a binocular microscope, and transferring them on the point of the needle into Carnoy's fixative. Puncturing the chorion while the egg is in fixative is not satisfactory, since the egg rapidly collapses, owing to extraction of water by the anhydrous fluid. For the preparation of sections I have in all cases used celloidin-embedded material. Whole embryos stain well with Auerbach's methyl-green acid-fuchsin mixture, the green-stained embryo contrasting well with the red-stained yolk; for staining sections the ordinary iron-haematoxylin method is satisfactory.

OBSERVATIONS.

In *Campodea* the 'dorsal organ' appears relatively much later than in *Symphyla* and *Collembola*.

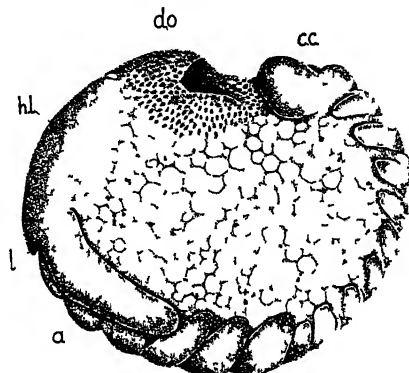
In embryos with the germ-band already clearly defined, and with the rudiments of cephalic and thoracic appendages present, the provisional body-wall appears as a fairly uniformly thin membrane, with rather closely approximated nuclei, through which the yolk, which it closely invests, is readily visible. In these early embryos the 'dorsal organ' is not present.

In the earliest embryo that I have secured with a recognizable rudiment of the organ (Text-fig. 1) the abdomen is already fully segmented, the entire complement of abdominal appendages has appeared, and the stomodaeal and proctodaeal invaginations are present. The rudiment of the 'dorsal organ' forms, in this embryo, a very conspicuous deeply and irregularly indented sheet of closely packed cells covering in the yolk from above, between the cephalic lobes and the tip of the abdomen; the provisional body-wall which laterally invests the yolk has, at the same time, become reduced to an excessively fine membrane with only sparsely scattered nuclei.

I have not been able to secure any embryos in which the transitional stages between the complete absence of the 'dorsal organ' and this well-defined rudiment were recognizable. The appearance of the organ and the simultaneous thinning out of the provisional body-wall suggest, however, a concentration of the cells towards the median dorsal pole, and support the statement of Uzel that the 'dorsal organ' in *Campodea*

arises by the drawing together of most of the cells of the provisional body-wall on to the dorsal surface of the yolk.

The manner of development of the 'dorsal organ' of *Campodea* therefore differs notably from that of both *Collembola* and *Symphyla*, where the organ arises by the enlargement of



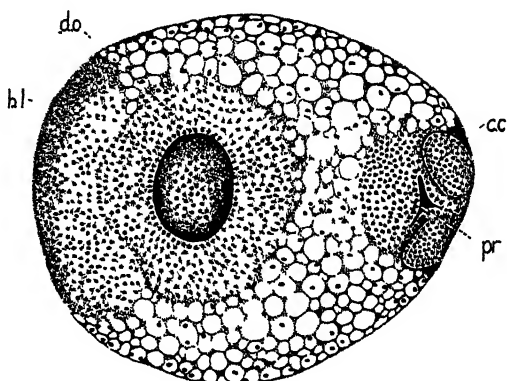
TEXT-FIG. 1.

Lateral view of an embryo, showing the 'dorsal organ' in an early stage of formation. The yolk is visible through the transparent provisional body-wall. The nuclei that are visible from the surface in the dorsal organ and in the provisional body-wall, but not those of the germ-band itself, have been indicated in the drawing. *a*, antenna; *cc*, caudal cercus; *do*, 'dorsal organ'; *hl*, head-lobe; *l*, labrum.

a circular patch of cells in the blastoderm, even before there is any trace of germ-band, and provides, indeed, the first clue to the dorsal pole of the egg.

A later stage in the development of the 'dorsal organ' is shown in Text-fig. 2. The drawing represents a dorsal view of a rather more advanced embryo than the foregoing, shortening of the germ-band having already begun. The organ is now closely associated with the cephalic lobes, rather than with the tip of the abdomen, and as contraction of the germ-band proceeds, this association between the two becomes more and more pronounced. The 'dorsal organ' still spreads over a large area of yolk. It has, however, assumed a more regular form, having become converted into a kind of flattened bowl with a wide

but shallow depression in the middle. Part of a section through this embryo, which was subsequently cut, is shown in fig. 1,



TEXT-FIG. 2.

View, from above, of an embryo showing the 'dorsal organ' with the initial stage in the formation of its cavity. All the nuclei visible from the surface, including those of the germ-band itself, have been indicated in the drawing. *cc*, caudal cercus; *do*, 'dorsal organ'; *hl*, head-lobe; *pr*, proctodaeum.

Pl. 1. The cavity of the 'bowl' is seen to be filled with a coagulated fluid. The floor of the 'bowl' is formed by cells that are already markedly larger than those which compose the provisional body-wall, and some of these enlarged cells have begun to taper, at their free ends, into short outgrowing processes. Even at this early stage of development degenerated nuclei have appeared, as small spherical deeply chromatic globules, among the cells of the 'dorsal organ'. They are eventually ejected from the organ, and become much more abundant as development proceeds (cf. fig. 4, Pl. 1).

From now on, the organ begins to intrude more deeply into the underlying yolk, its form becoming gradually more spherical while its orifice slowly contracts (cf. figs. 2, 3, and 4, Pl. 1). At the same time it comes to occupy a progressively smaller area on the dorsal surface of the yolk, the mature organ being much less conspicuous than in earlier stages of its development (cf. Text-figs. 2 and 3).

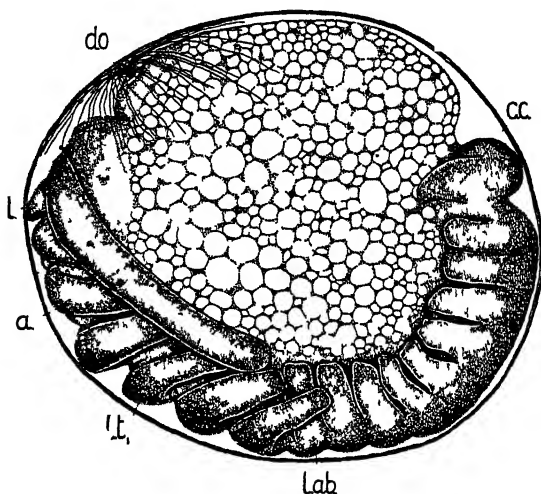
These changes are accompanied by the outgrowth of fine filaments from the cells of the floor of the organ into its cavity. The first sign of the formation of these filaments is already to be seen in the 'dorsal organ' shown in fig. 1, Pl. 1. As they elongate they form at first a loose tangle of short threads in the cavity of the organ (fig. 2, Pl. 1). But as the organ begins to assume its definitive form they collect into a bundle and grow out through the gradually narrowing orifice into the space between the embryo and the blastodermic cuticle (fig. 3, Pl. 1).

The latter is a thin transparent colourless membrane, which adheres loosely to the chorion. It is readily seen when the egg is cut in section, and, in broken eggs, can be separated from the chorion by fine needles. It is not directly connected with the rim of the 'dorsal organ', as in *Symphyla*, but, as in *Collembola*, forms a closed sac within which the embryo is contained. But it does not, as in *Collembola*, become a secondary functional egg-shell, for in *Campodea* the precocious rupturing of the chorion, so commonly seen in *Collembola*, does not take place.

In the embryos at the stage shown in Text-fig. 3, the 'dorsal organ' has attained its full development. A section through the organ (from an embryo cut in the sagittal plane) is shown in fig. 4, Pl. 1. It is now almost spherical, and intrudes well into the underlying yolk. The filaments, which number about 70-80, have collected into a narrow axial bundle that emerges through the orifice. The cells from which these filaments arise occupy mainly the floor of the organ. They are closely crowded, and, since their nuclei lie roughly in two layers, present the misleading picture of a two-layered epithelium. Although much larger than the cells of the surrounding ectoderm, they are smaller than in *Collembola* and *Symphyla*. The texture of their cytoplasm is also less suggestive of a glandular function; it is not markedly granular, and though it may, in some preparations, appear faintly reticulate, is not heavily vacuolated. Towards the orifice of the organ are a number of large cells, but no fibrils originate from them. At the surface the cells are more flattened.

The course of the filaments, after these emerge from the orifice of the organ, can be examined only in whole embryos,

with chorion intact. I have had much greater trouble in following the filaments individually than in *Symphyla*, and even



TEXT-FIG. 3.

Lateral view of an embryo, showing the 'dorsal organ' at the height of its development. The chorion and blastodermic cuticle have been included in the drawing. *a*, antenna; *cc*, caudal cercus; *do*, 'dorsal organ'; *l*, labrum; *lab*₁, first abdominal leg; *lt*₁, first thoracic leg.

Collembola. The difficulty seems to arise partly through the need of examining them through the intact thick brown chorion; but to this must be added their extreme delicacy, for they are much finer than the relatively coarse filaments of *Symphyla*. In cases where the blastodermic cuticle lies close against the surface of the embryo, they are, indeed, almost unrecognizable, and are to be seen clearly only when the embryo has shrunk away a little from the cuticle. They are then to be seen radiating away from the organ, as in *Collembola* and *Symphyla*. They seem, however, to be much shorter than those of *Collembola*, and even in the best preparations that I have obtained show no evidence of approaching the equator of the egg.

The 'dorsal organ' survives up to the time that the ventral

flexure is completed. In place of the provisional body-wall investing the yolk the definitive body-wall, with indication of intersegmental lines, has now formed. The 'dorsal organ' is then seen to be located within the first thoracic segment, or probably more precisely within the membrane between that segment and the head.

Shortly after the completion of the ventral flexure the 'dorsal organ', despite its relatively late appearance, begins to degenerate. An early stage in the degeneration is shown in fig. 5, Pl. 1. The drawing represents a sagittal section along the anterior end of an embryo that has recently gone into ventral flexure. Though rather shrunken the organ still maintains its general form; but the cytoplasm of the cells has acquired a thick gelatinous consistency, and individual cells are scarcely distinguishable. The nuclei now mostly stain very heavily, the individual chromatin grains are no longer visible, and the nuclei are obviously in course of degeneration. The axial bundle of filaments is still present, but the degenerating filaments have begun to fuse with one another into a viscid mass. The degenerating organ does not lie within the lumen of the developing mid-gut, as in *Collembola*, but, as in *Symphyla*, disrupts within the haemocoel.

A later stage in the process of degeneration is shown in fig. 6, Pl. 1. The entire organ has become flattened out under the dorsal epidermis, and its lumen has disappeared. The filaments are no longer visible. Most of the degenerated nuclei are still seen as irregular clumps of deeply chromatic material. Even the cells at the surface of the organ, judging by the depth of staining of their nuclei, seem to participate in the degeneration.

Finally, with the advance of new epithelial cells from the surrounding epithelium, the 'dorsal organ' becomes closed in from above and completes its degeneration within the haemocoel (fig. 7, Pl. 1). It gradually dwindles in size. The degenerated nuclei form deeply chromatic spheres which become ejected from the disrupting cells, and long before the larva is ready to emerge from the egg all trace of the organ has vanished.

DISCUSSION.

There can be little doubt that in the 'dorsal organ' of *Campodea* embryos we have a structure which is strictly homologous with the organ of the same name of symphylid and collembolan embryos (Tiegs, 1940 and 1942). It undoubtedly shows some differences, notably the manner of its formation, its relatively brief life, and its weaker development; but these differences cannot obscure its obvious affinity with the 'dorsal organ' of these groups.

In the embryo of another member of the Entognatha, *Japyx*, a 'dorsal organ' was described by Grassi as long ago as 1885. It would be of importance if it could be shown to be an organ of the same type as that of *Campodea*. In the damp rain-forest country of Victoria a species of *Japyx* (*J. leae*) is not infrequently encountered in decaying wood and in moist soil. The eggs are, however, very difficult to obtain, for they are usually deposited in soil at a depth of six inches or even more. Like those of *Campodea* they are deposited in small clumps, but when found can always be identified by the presence of the female that mounts guard beside them. Several batches of eggs have been obtained for me by Dr. F. H. Drummond from soil under a log in which the insects had been detected. In one of these batches the embryos within the eggs are in the dorsally flexed germ-band condition, with fully segmented abdomen, full complement of appendages, and well-formed caudal forceps. At a corresponding stage of development in *Campodea* the 'dorsal organ' is at the height of its development. Yet in the *Japyx* embryos there is no sign of the organ. I have also a number of embryos of the giant Australian *Heterojapyx gallardi* given me by the late Dr. R. J. Tillyard. These eggs are very large, measuring as much as 1.6 mm. in diameter. In several of these the embryos are at the stage in which, in analogy with *Campodea*, a fully developed 'dorsal organ' might have been expected; yet, as in the *Japyx* embryos, the organ is quite absent. The evidence from *Campodea*, *Japyx*, and *Heterojapyx* seems to suggest that in the Entognatha the 'dorsal organ' is in process of retrogression.

The common possession of so distinctive an organ by Symphyla, Collembola, and Campodea points to a close affinity between these groups and lends weight to the theory of a derivation of insects from Symphyla-like ancestors; Collembola admittedly are not accepted as insects by all writers, for they present many anomalies, but Campodea is, by general consent, a true insect.

In no other insect whose development has hitherto been examined is there any certain evidence of a 'dorsal organ' of the Symphyla type. The so-called 'dorsal organ' of pterygote insect embryos is merely a stage in the involution of the embryonic membranes; to quote Wheeler (1889) who first used the name in this sense, 'the term "dorsal organ" has been applied to the peculiar thick clump of cells resulting from the concentration on the dorsal yolk of the remains of either the amnion or serosa, or of both, preparatory to their sinking into the yolk or being absorbed'. There can be no justification for drawing an homology between this so-called 'dorsal organ' (and of the serosa) with the 'dorsal organ' of collembolan embryos, as Heymons (1901) has done; for in Collembola, as in Symphyla and Campodea, the structure in question is not a mere local accumulation of cells as was once believed, but a highly differentiated and specific embryonic organ having nothing in common with a serosa. The name 'dorsal organ' (primary dorsal organ) has also been applied by some authors to a median dorsal thickening that appears in the ectoderm above the yolk in the embryos of some pterygote insects (references are given in my earlier paper; cf. also Hirschler, 1928). In the absence of any structural differentiation it is impossible to assess the meaning of this structure. It may be the vestige of a true 'dorsal organ'; but in that case it is surprising that there is no reference to such an organ in the embryo of *Machilis* or *Lepisma* or of any Orthopteran hitherto examined.

In the extensive literature on arachnid and crustacean embryology there are many references to 'dorsal' and 'dorso-lateral organs' (some references are given in my previous paper). The structures in question are variously described as

glandular, adhesive, &c. The descriptions hitherto given of these organs do not suggest any obvious affinity with the 'dorsal organ' of Collembola, Symphyla, and Campodea. It is, however, desirable that these organs, especially in Crustacea, should be more carefully examined; for the discovery of a 'dorsal organ' of the Symphyla type in a crustacean embryo would go far to invalidate the evidence that the 'dorsal organ' offers for an immediate affinity between Symphyla and insects.

There remain for consideration the Myriapoda. Amongst the diplopods, whose embryology has been frequently investigated, there is no known case of a 'dorsal organ'; and so conspicuous a structure, were it present, could hardly escape detection. A more extensive search amongst the diplopods might, nevertheless, reveal its presence in some forms, for there seems little doubt as to the close relation of these myriapods and Symphyla. Special significance seems to attach to the Pselaphognatha, for these are commonly regarded as the most primitive of the Diplopoda. Metchnikoff (1874), who made some observations on the early development of *Polyxenus*, makes no mention of a 'dorsal organ'. I have obtained a large number of rather late embryos of an undescribed Australian species of *Polyxenus*; there is no trace of a 'dorsal organ' in these embryos, nor any recognizable remains of one that might have been present in earlier embryos.

In *Paupopus*, whose embryology has not yet been described, a 'dorsal organ' is present. I have obtained a large series of eggs of an Australian species, of whose development I hope to give an account later. It will suffice here to state that the organ in question is not of the type found in Symphyla, Collembola, and Campodea, for the filaments are not present; it produces, instead, a secretion which spreads for a short distance under the blastodermic cuticle and probably has an adhesive function.

Turning to the Chilopoda we find a single reference to a 'dorsal organ', Heymons (1901) having reported its presence in *Scolopendra*. In the embryo of this myriapod it forms a large crescentic thickening in the membrana dorsalis (pro-

visional body-wall), just behind the developing head. Other crescentic wrinkles form when the embryo proceeds into the ventral flexure, but these soon disappear again. The 'dorsal organ' outlasts these; but its cells eventually degenerate, and in the advanced embryo all trace of it is lost. It is difficult to assess the significance of this structure. In embryos of *Geophilus*, which I have been able to examine at a corresponding stage of development, such an organ is not present; and it is also noteworthy that neither Metchnikoff (1875), nor Zograff (1888), refers to it in the embryos of this chilopod. I have not been able to obtain any *Scolopendra* embryos for comparison; but in a local related chilopod (*Cormocephalus esulcatus*), of which I have been able to obtain several batches of embryos in the ventrally flexed condition, there is no indication of such a structure. At present the evidence from *Scolopendra* is too inconclusive to justify comparison with the highly differentiated organ of Symphyla, Collembola, and Campodea.

The function of the 'dorsal organ' is still obscure. In Symphyla and Collembola its structural relations are at least consistent with the suggestion that it may be a water-absorbing organ (Slifer, 1938); for if the inner cuticular sheath (blastodermic cuticle of Symphyla; second cuticle of Collembola) is impermeable to water, and if the extra-embryonic filaments are hygroscopic, then the organ would serve as a ready means for conducting to the embryo moisture that had passed through the egg-shell. In the case of Campodea, however, the need of such an organ is not evident for there is no inner cuticular sheath comparable to that of Collembola; and the entire system of embryonic filaments is surprisingly short. Until some means have been found of subjecting these very minute eggs to experimental tests judgement on the question must be suspended. Perhaps the organ will ultimately be found to have merely some attaching function.

SUMMARY.

1. Although showing differences in the manner and time of its development, the 'dorsal organ' of Campodea embryos

is essentially the same type of organ as that of Symphyla and Collembola.

2. This must point to a close affinity between Symphyla, Collembola, and Entognatha; for in no representatives of any other group of Arthropoda has such an organ been found. Even in the apparently closely related Diplopoda there is no record yet of its presence, while in *Pauropus* the 'dorsal organ' is of a different type.

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EXPLANATION OF PLATE 1.

bc, blastodermic cuticle; *br*, brain; *c*, chorion; *dbw*, definitive body-wall; *degn*, degenerating nuclei; *do*, 'dorsal organ'; *fb*, fat-body; *mg*, mid-gut; *oes*, oesophagus; *pbw*, provisional body-wall; *yn*, yolk-nuclei.

Fig. 1.—Transverse section through developing 'dorsal organ' of the embryo shown in Text-fig. 2. $\times 380$.

Fig. 2.—Similar section from a more advanced embryo, showing an early stage in the formation of the filamentous outgrowths. $\times 570$.

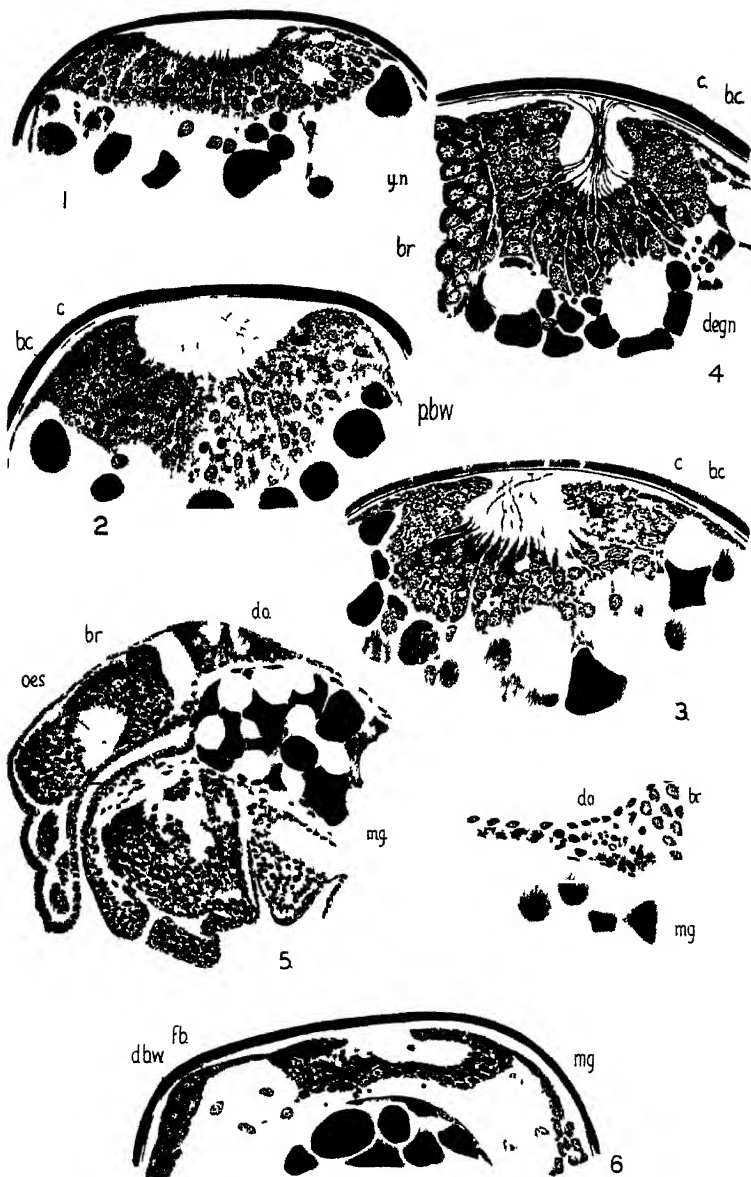
Fig. 3.—Similar section; the filaments have begun to grow through the gradually narrowing orifice of the 'dorsal organ' into the space between the embryo and the blastodermic cuticle. $\times 760$.

Fig. 4.—Fragment of a sagittal section along the mid-dorsal surface of an embryo, at about the stage shown in Text-fig. 3. The 'dorsal organ' is now at the height of its development. The mass of filaments has contracted into a narrow axial bundle. The apparent ending of the filaments shortly after emerging from the cavity of the organ is due to their being cut by the microtome knife. $\times 570$.

Fig. 5.—Sagittal section along the anterior end of an embryo, shortly after completion of the ventral flexure; chorion and blastodermic cuticle removed. It shows the beginning of degeneration in the 'dorsal organ'. The nuclei of the latter have mostly become very deeply staining; in the axial bundle the individual fibrils are no longer distinguishable. $\times 380$.

Fig. 6.—Degenerating 'dorsal organ', from a transversely cut embryo. Most of the nuclei are in an advanced state of degeneration. The axial bundle of filaments is no longer present. The definitive body-wall is beginning to encroach upon the degenerating 'dorsal organ'. $\times 570$.

Fig. 7.—Fragment from a sagittal section along mid-dorsal surface of a more advanced embryo, showing a late stage in the disruption of the 'dorsal organ' (anterior end to right; chorion and blastodermic cuticle removed). The 'dorsal organ' has now become greatly reduced in bulk and has become overgrown by definitive epidermis. $\times 760$.



Micro-anatomical Studies on Asellus.

By

A. E. Needham, M.A., B.Sc.

With Plate 2, and 8 Text-figures.

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1. Autotomy, p. 49. 2. Oostegite, p. 57. 3. Microtrichs, p. 59. 4. Pigment pattern, p. 64. 5. Asellus aquaticus and Asellus meridianus, p. 67.

1. THE AUTOTOMY MECHANISM OF THE THORACIC APPENDAGES

Introduction.

THE power of autotomy is developed in the thoracic limbs of Asellus, as well as in other appendages (Zimmer, 1927, p. 749). It is interesting to find that in the adult male the appendages of the fifth thoracic segment, specially modified to grasp the female in mating (Text-fig. 1 B), do not autotomize at all readily. When, occasionally, they do break, the fracture is ragged and does not follow exactly the normal fracture line. The normal function of autotomy, well developed in this pair of appendages in the female and in the young of both sexes, has apparently been lost in the mature male as an adaptive feature: the loss of either member of the pair would render the male incapable of holding the female. It seemed worth investigating whether or no this absence of autotomy has an anatomical basis. Sections (8μ thick) were cut through the autotomy region of the fifth and sixth thoracic appendages of the male Asellus, and of the fifth of the female, longitudinally to the limb axis and transversely to the body. The celloidin double-embedding method was used and Mallory's triple stain.

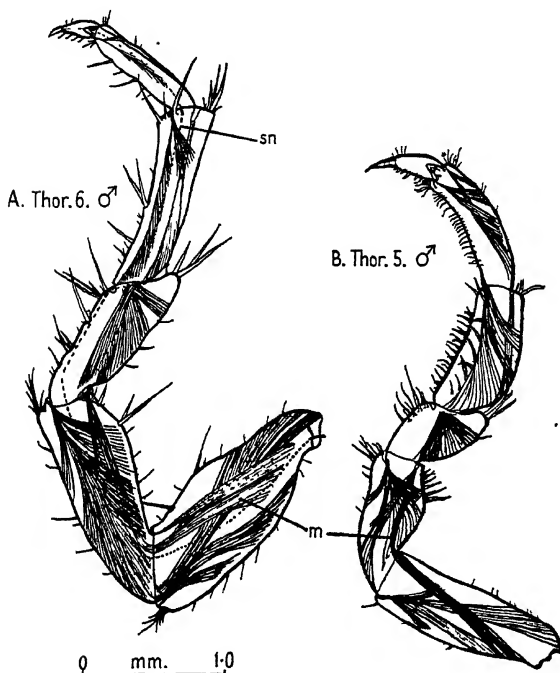
Observations.

The micro-anatomy of the normal autotomy mechanism in Asellus (e.g. thoracic 6 of male) does not appear to have been described. It shows similarities to that of Decapoda (Andrews,

1890; Pearson, 1908; Emmel, 1910; Paul, 1915) but on a smaller scale. The membrane (Text-fig. 2, *mb*) which closes the cavity of the limb except for a small aperture ventrally, through which the segmental nerve (*sn*) and afferent and efferent blood channels (Text-fig. 3, *bv*) pass, consists of a few cells only, and perhaps differs from that of some decapods in being single-layered and entirely proximal to the fracture plane (fig. 1, Pl. 2); in Insecta Orthoptera (Bordage, 1905), as well as in Decapoda (Andrews, Pearson, l.c.) the membrane has been described as double, with the fracture plane situated between the two layers. The cells of the membrane in *Asellus* (fig. 1, *mb*, Pl. 2) contain cross-striated fibrils which contract after autotomy and help to close the wound. They are inserted on to the exoskeleton, but appear to pull away on contraction. Connective tissue accompanying the blood channels is attached to the membrane on both surfaces (fig. 1, *ct*, Pl. 2), and may conceivably help to occlude the blood space (cf. Emmel, 1910). The fracture plane lies near the proximal end of the fused basipodite and pre-ischiopodite (Hansen, 1925). The two thick, inner layers of the exoskeleton, which stain blue with Mallory's stain, are interrupted here by an annular fissure filled with a substance which stains red like the thin superficial layer of the exoskeleton (fig. 1 A, Pl. 2). It may perhaps be legitimate to refer to these three layers of the exoskeleton as endocuticle, exocuticle, and epicuticle, as in insects (Wigglesworth, 1939, p. 17). The epidermis cells provide no evident basis for the failure of deposition of endo- and exocuticle in the narrow fissure; in *Astacus* there appears to be a band, several cells wide, of more lightly staining epidermis associated with the fissure. The epicuticle-like substance in the fissure must be introduced from within, since epicuticle is the first layer of the exoskeleton to be laid down after moulting, and thus seals the fissure on the outside.

It seems probable that normal autotomy is a reflex action in *Asellus*, as in other animals. It occurs more readily when the limb is lightly held than if it is gripped sufficiently firmly to paralyse the muscles of the distal segments, and it occurs without any bending of the limb by the operator. Denervated

limbs are not autotomized. In the act of autotomy the ischium is raised, making an acute angle dorsally with the pre-ischium (Text-fig. 1), and then suddenly straightened. Fracture appears



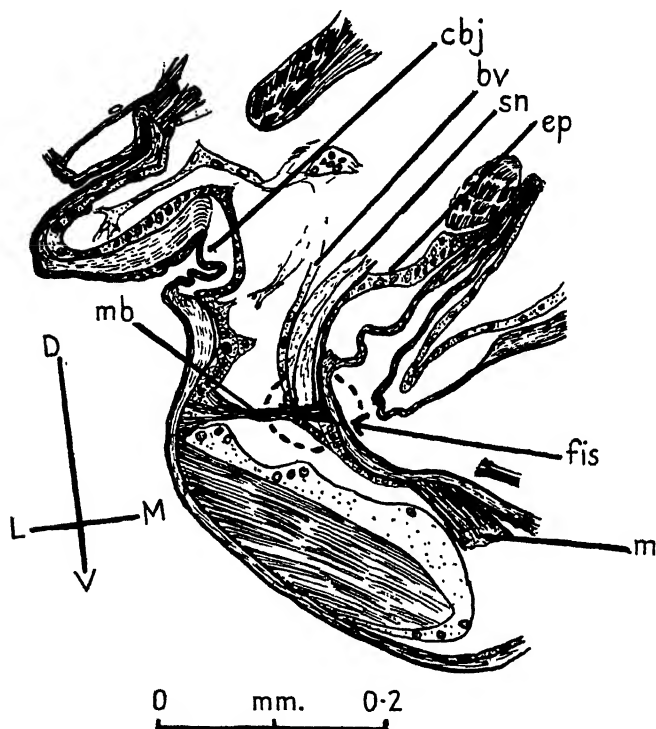
TEXT-FIG. 1.

Thoracic appendages of male *Asellus aquaticus*, posterior view. A, Thoracic 6; B, Thoracic 5. (In B the levator of the ischium is hidden by the muscle *m*.)

For explanation of lettering of this and other text-figures see p. 72.

to occur on straightening (*cf.* Tschetwerikoff, 1910, p. 427). The muscle *m* (Text-fig. 1) which, unlike the other limb muscles, traverses two segments, seems well adapted for this purpose and would initiate a fracture ventrally, where the fissure is better developed than on the dorsal side. As the nerve and blood-vessels are situated near the ventral side (Text-figs. 2, 3)

they are readily broken across, but there is no indication of a preformed fracture plane in them. Autotomy occurs most readily if the limb is held in the carpus region, which supports the supposition that the muscle *m* is essentially concerned.



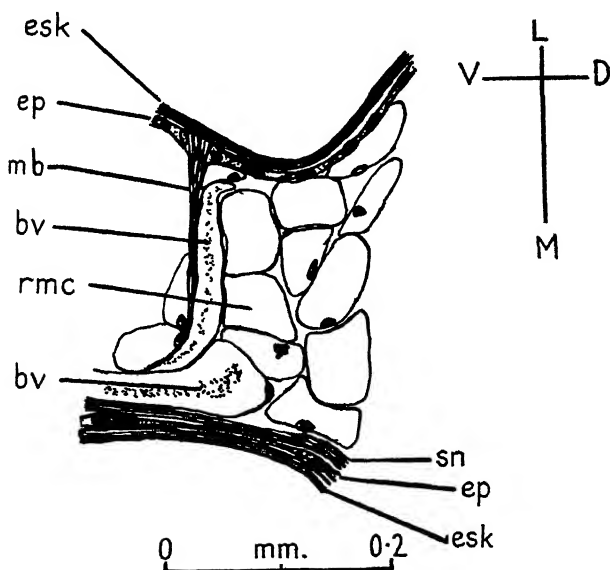
TEXT-FIG. 2.

Longitudinal section through autotomy region of thoracic appendage of young *Asellus aquaticus*, transverse to axis of body. The dotted line encloses the region shown (from an adult) in fig. 1, Pl. 2.

Two small muscles, dorsal and ventral, are inserted on the exoskeleton just proximally to the fracture plane, and originate in the coxa. They possibly help to withdraw the stump of the limb from the overlying exoskeleton after autotomy (p. 50): this withdrawal is certainly very marked.

In the fifth thoracic limb of the adult male all essential

features of the autotomy mechanism are present (fig. 1 B, Pl. 2; Text-fig. 1 B). The only obvious anatomical difference from the normally autotomizing appendage is the almost complete obliteration of the fissure in the endo- and exocuticle. The fact that enforced breakage (p. 49) does not follow exactly



TEXT-FIG. 3.

Longitudinal section through autotomy region of thoracic appendage in an older specimen of *Asellus aquaticus* showing blood-vessels and reserve material cells.

the fissure suggests that this obliteration is the important factor. (Other differences between figs. 1 A and B, Pl. 2, are due to the fact that they are not taken from exactly corresponding sections of their respective limbs.) In the adult female the mechanism in the fifth thoracic appendage is that of a normally autotomizing limb. Even in the adult male the fifth thoracic appendage will sometimes autotomize, especially in winter, when breeding is at a minimum.

The uropods are the most easily autotomized appendages in

Asellus. The antennae and antennules are also readily cast; pleopods 2 of the female and the gills (pleopods 3-5) also autotomize fairly readily. Wege (1911) has investigated the second antenna but the others have probably not been studied in detail. The mouth parts and pleopods 1 and 2 of the male are not normally autotomized but they regenerate if removed (cf. Needham, 1941). Regeneration of thoracic limbs occurs even as early as stage 5 of the embryo (Needham, 1942).

Discussion.

It is surprising that the anatomical difference which determines the non-autotomy of an appendage so vital as the fifth thoracic limb of the male *Asellus* should be so slight, and yet so effective. The possibility of a physiological difference, in addition, must be envisaged, but the result of enforced breakage (p. 49) suggests that the anatomical difference alone may be sufficient.

The apparent difference between the normal mechanism in *Asellus* and that of Decapoda and Insecta (p. 50) calls for comment. It is curious that, in those forms, the fracture plane should be situated between the two layers of the membrane, since there can be little adaptive reason for the closure, by the distal layer, of the end of the discarded limb. In the chela of *Astacus* there is a superficial appearance of two membranes arranged in this way, but a closer examination shows that the distal 'membrane' is nothing more than the sudden cessation, in the region of the fracture plane, of spongy connective tissue which fills the whole cavity of the limb except for the blood channels. Proximally to the fracture plane, likewise, the connective tissue ends abruptly, but here it is considerably strengthened, particularly towards the anterior and posterior aspects of the limb, by fibres (which appear to be elastic rather than muscular as in *Asellus* (p. 50)). They are inserted on to the exoskeleton between the epidermis cells, and histologically resemble the rest of the connective tissue, except that they have a specific orientation; the whole membrane is therefore probably mesodermal in origin (cf. Paul, Pearson, Andrews, l.c.). The efferent blood sinus is very wide, filling the centre of the limb

cavity, so that the membrane is more or less completely divided into anterior and posterior portions, which may correspond to the two 'valves' of other decapods (Emmel, 1910; Paul, 1915). Pearson (1909) describes the double membrane of *Cancer* as epidermal, while Andrews (1890) found in addition a double ingrowth of exoskeleton in the crab *Libinia*.

The fissure of *Astacus* resembles that of *Asellus*, and is likewise better developed ventrally than on the dorsal side of the limb. Here the fissure contains epicuticular substance, while on the dorsal side the layers of the exoskeleton meet and almost obliterate the fissure. The transition from the functional to the occluded region of the fissure is quite abrupt. In the fifth thoracic appendage of the male *Asellus* the whole fissure is occluded, and it seems probable that in all cases a functional fissure is formed all round the limb, initially, and is later obliterated, on the one side only, or completely. In *Astacus* the fissure, where it is functional, does not pass completely through the exo- and endocuticle but leaves a thin layer both externally and internally. The internal of these layers is more or less separated, on the distal side of the fissure, from the rest of the exoskeleton by irregular spaces, containing what appear to be remains of cells; these cells may possibly be concerned in maintaining the open cavity of the functional fissure: their remains stain the same shade of red (a combination of orange-G and acid fuchsin) as the normal substance in the fissure (p. 50), and as the epicuticle. The irregular spaces mentioned are in open communication with the fissure and are less well developed on the dorsal side where the fissure is obliterated. There is no indication of two fissures, a proximal and a distal, as Reed (1904) has described for the crayfish and hermit crab, and Andrews (1890) for *Libinia*.

The evidence that the fracture plane represents an original intersegmental joint in *Asellus* (p. 50) is perhaps insufficient, though the small muscles (p. 52) would seem to lend additional support to this theory. Andrews (l.c.) does not consider this to be the case in the crayfish or the lobster, and in the chela of *Astacus* there is a vestige of an original basi-ischial joint quite separate from the autotomy fissure. On the other hand,

the joints of Crustacea are characterized by the absence of the intermediate layers of the exoskeleton (Text-fig. 2, *cbj*), leaving merely the outer (epicuticle+exocuticle) and innermost layer (of endocuticle). In the autotomy fissure the region from which the intermediate layers are absent has been reduced to a minimum. From the condition at the intersegmental joints it would seem that flexibility depends on the exocuticle. If so the Crustacea differ radically from the Insecta (Wigglesworth, 1939, p. 18) where the exocuticle appears to be the more rigid, and is therefore reduced at the joints, and (to produce an essential weakness) along the 'ecdysial line' (*ibid.*, p. 28). In the Crustacea the endocuticle has probably become the more rigid through the deposition of inorganic salts.

It seems probable that in the male *Asellus* non-autotomy of the fifth thoracic appendage, correlated with active breeding, may be under the control of sex hormones as in the case of the oostegites of the female (p. 57). It must be supposed that following a certain 'maturity moult' the autotomy fissure is not maintained in a functional condition.

The method of autotomy in decapods has been described by Fredericq (1892), Andrews (1890), and Wirén (1896). The limb is usually (but not in all forms) raised until it rests against the carapace, distally to the fracture plane. With this as a fulcrum the fracture is said to be caused by the contraction of the extensor (levator) of the segment (basi-ischium) in which the fracture plane is situated, and to begin therefore on the dorsal side of the limb. Wirén, however, believes the extensor of the merus, i.e. a muscle distal to the fracture plane, to be the effective one. This muscle originates on the ventral side of the basi-ischium and so fracture would begin ventrally as in *Asellus*, and presumably in *Astacus* also. If a muscle proximal to the fracture plane also participates, it is presumably the flexor (depressor) of the basi-ischium.

In *Asellus* the corresponding muscles (of the basi-preischium in this case) are not arranged as in decapods, but in the form of antagonistic rotators (p. 64). Again, there is in *Asellus* no fulcrum corresponding to the decapod carapace; but it seems possible that the dorsal side of the joint between

basi-pre-ischium and ischium, when held by the levator of the ischium in the position shown in Text-fig. 1, acts as a pulley for the muscle *m*, increasing its effective force.

2. EXPANSION OF THE OOSTEGITES IN THE FEMALE.

Introduction.

The oostegites, which form a brood pouch in the breeding female of all Peracarida, develop as small horizontal projections from the median face of the coxopodite of the anterior thoracic (2-5) limbs. In *Asellus* they grow slowly in the young female and attain functional size suddenly at a single moult (Unwin, 1920; Rosenstadt, 1888) which occurs during coition, after which fertilized eggs are passed into the brood pouch. The release of the brood in 4 to 6 weeks is followed by another ecdysis, when the oostegites return almost to their immature form and size. This cycle of behaviour is repeated for each brood. The anatomical basis of this cycle is of some interest.

Observations.

Fig. 2 A, Pl. 2, shows a section of an unexpanded oostegite and figs. 2 B and c, Pl. 2, of the expanded condition, the latter (c) near the base of the oostegite and the former (B) near the edge. The idea of unfolding, expressed by Maercks (1931, p. 403) and van Emden (1922) is misleading, the unexpanded organ being a comparatively thick blade without any folding, and the expansion is largely effected by reduction in thickness, the thickness of actual tissue being only about one-sixth of that in the unexpanded blade. In this process the epidermis cells become drawn out from their columnar form, and the enclosed blood spaces (*bs*) compressed. Fibrillar strands (*fs*) spanning the cavity appear to contract, since they are shorter but still straight in the expanded oostegite, and they probably play a part in expansion, assisted by turgor of the organ. It seems possible that pressure by the blood may be involved, as in the expansion of the wings of the insect imago; there appear to be an afferent vessel antero-dorsally (*bv*), and a wide efferent sinus postero-ventrally.

Other changes accompany the expansion. The pigment cells, (*pic*) originally rather deeply situated, come to spread out in a functional position below the epidermis. Certain large cells below the epidermis (*rmc*) become much less deeply staining, and their large vacuole decreases in volume. Many of the epidermal cells likewise lose their original reserve material. It seems possible that this is used in the secretion of exoskeleton, after the expansion. The exoskeleton, originally very thin as compared with that of the general body surface, becomes very much thicker on the outer, ventral, side of the lamina. The reserve material cells appear particularly depleted on this side. The thickened chitin is presumably protective. The inner wall is probably thin enough to permit respiratory or other chemical exchange, but the epithelium below it is unlike typical epithelia performing such functions (e.g. gills).

From fig. 2 B, Pl. 2, it is clear that a considerable extent of the oostegite round the periphery is exoskeleton only, containing no living tissues. There is, however, every reason to believe that chitin is laid down only where cells are in direct contact, and it seems probable that the tissues at first fill the expanded oostegite completely but withdraw from the periphery during incubation of the brood, thus facilitating the return to an immature condition at the next ecdysis. The gradual decrease in thickness of the exoskeleton of the outer surface, from the centre towards the edge of the blade, suggests a progressive withdrawal of the skeleton-secreting epithelium from peripheral regions, supposing the deposition of exoskeleton to continue throughout the intermolt period wherever the epithelium remains in contact with it. It is, however, possible that the greater thickness of living tissue centrally, at the outset, may be partly responsible for the difference in thickness of the exoskeleton. The withdrawal of tissues is not an artefact of fixation and may be observed in living oostegites. Complete return to the immature form is only possible after ecdysis.

The functional oostegites are capable of movement to expand the brood pouch and aerate the brood, to rearrange the latter and eventually to release it. There are dorso-ventral muscle strands attached to the outer (ventral) border of the base of the

organ. Stimulation of an ovigerous female in the region of the anterior opening of the brood pouch causes premature release of the brood, and is elicited with increasing facility as incubation approaches full term.

The cyclic behaviour of the oostegites occurs throughout the summer, and is usually associated with the deposition of eggs into the brood pouch, whether or no copulation takes place. In *Porcellio scaber*, on the other hand, according to Schobl (1880), failure of fertilization on one side of the body, owing to obstruction of the genital pore, results in a failure of the oostegites on that side to expand.

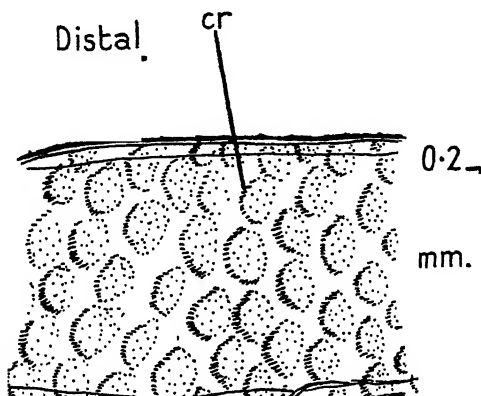
3. THE MICROTRICH SYSTEM.

Apart from the setae proper ('macrotrichs'), which have been described in some detail by Tschetwerikoff (1910) there is, in *Asellus*, a well developed system of fine hairs of a much smaller order of magnitude (approx. 1.8μ long). Tschetwerikoff (l.c., p. 469) probably noticed them on the maxillipeds where they are specially long, and Racovitza (1919) figures them on the copulatory appendages of the male (Racovitza, 1922, 'écailles frangées', &c.). It is probable (cf. Racovitza, 1922) that the system is universally distributed throughout the Crustacea, though not always so well developed as in *Asellus*. There seems no reason to doubt that it corresponds to the 'microtrich' system of insects (Wigglesworth, 1939, p. 17).

Appropriate stains (e.g. aniline blue) are necessary to show the microtrichs clearly, though they may be seen even in the unstained exuviae, by careful focusing under an oil-immersion objective. Typically they are arranged in short crescentic rows, in *Asellus* (Text-fig. 4), evenly distributed all over the body. From the base of each hair a minute cone of deeply staining skeletal material extends into the body for a distance far exceeding the length of the trichs (Text-fig. 6, *sc*). The true setae also possess these cones (Needham, 1941), which appear to be associated with a sensory function (cf. Wigglesworth, 1939, p. 131).

Each crescent stands on a slight ridge of the exoskeleton, and each corresponds to one area, usually hexagonal in shape (Text-fig. 5, *ca*), into which the exoskeleton is divided. These

areas (Tschetwerikoff, 1910), correspond to the underlying cells of the epidermis (cf. insects, Wigglesworth, 1939, p. 12). They measure about $14 \times 10 \mu$, and within each area many 'pore-canals' (Wigglesworth, l.c.) pierce the exoskeleton (Text fig. 6, *pc*). Towards the margin of each cell-area the pore canals



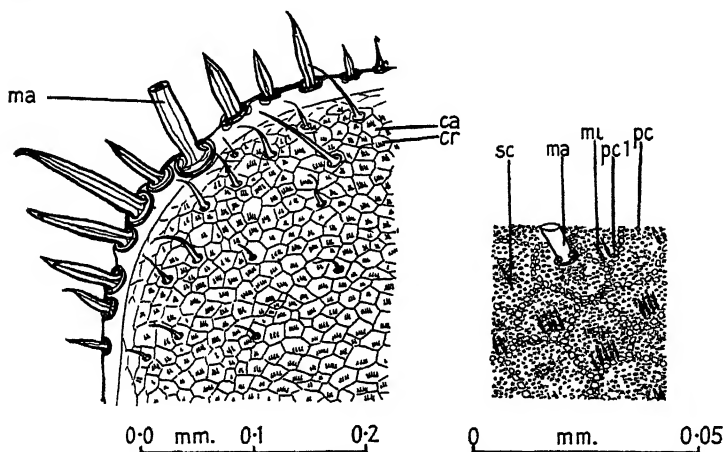
TEXT-FIG. 4.

Exoskeleton of most distal peduncular segment of second antenna of *Asellus meridianus* to show crescents of microtrichs.

become progressively smaller and fewer, but unusually large ones (*pc* 1) mark the division between neighbouring cells. The pore canals stain more lightly than the rest of the exoskeleton, and the crescent of trichs is set in a similarly lightly staining region of the exoskeleton, through which cell processes reach the trichs (cf. insects; Tiegs, 1922).

Where the exoskeleton is creased, the crescents tend to be straighter and follow the line of folding, and the cell areas are similarly elongated; e.g. on the terga of the anterior, coalesced segments of the abdomen, and on parts of the thoracic sterna. Conversely, on narrow tubular structures (e.g. rami of the uropods) they are drawn out along the ramus so that the arms of the crescent are almost parallel. The segments of the peduncle of the antenna, however, are wide enough for the crescents to maintain the typical form (Text-fig. 4).

The convexity of the crescents is everywhere directed outwards, that is: forwards on the anterior half of the body, backwards on the posterior half, laterally on the pleura, and distally on the appendages. The trichs themselves point in the same direction, making an acute angle with the body surface. The number of crescents and the number of trichs in each crescent decrease from the centre of the body to the margins (Text-fig. 5).



TEXT-FIG. 5.

TEXT-FIG. 6.

Fig. 5. Surface of postero-lateral angle of pleuron of thoracic segment of *Asellus meridianus* to show cell areas and microtrichs.

Fig. 6. Part of Text-fig. 5 enlarged to show cell areas, pore canals, and skeletal cones attached to the base of the microtrichs.

There are modifications of the system on certain appendages: Antennae: no trichs distally. Mandibles: none on biting surface, well developed elsewhere, specially long on palps. Maxillules: none on biting surface, stout combs on outer edge of distal endite, transverse to axis. Maxillae: trichs long distally on endites. Maxillipeds: elongated on basis and median face of endites. Labrum and Paragnaths: long and profuse especially near tip and median furrow of latter. Pleopods 1 of male: abundant medially, pointing medially. Pleopods 2 of male: endopod, none; exopod, abundant medially, sparse laterally,

pointing medially and laterally resp. Operculum (Pleopod 3): abundant proximally. Gills (Pleopods 4, 5): trichs absent.

Other Crustacea.

1. *Gammarus* (see Sexton, 1924, p. 356, 'microscopic spinules').—Trichs shorter than in *Asellus*. In broken lines (ridges) not crescents, more than one to each cell area. Towards periphery trichs shorter or completely absent and ridges flattened, but no reduction in number.

Distribution.—Head and dorsal body surface: ridges without trichs. Ventral body surface: still less developed. Thoracic appendages: typical proximally, no trichs distally. Mouth parts: greatly elongated on mandibular palp, endites of both pairs of maxillae, median edge of basis of maxilliped, tip of paragnaths and labrum. Gnathopods: specially well developed on merus. Pleopods and Antennae: reduced or absent. Uropods: well developed ridges.

2. *Balanus*.—General body surface: scattered elevations without trichs. Mouth parts: typical crescents. Cirri: the long fine hairs are probably microtrichs. Penis: regular annuli of trichs.

3. *Chirocephalus*.—Mid-ventral groove: crescentic lines of 'setules'. Thoracic limbs: proximal endites, very long trichs, conspicuous cell areas; distal endites, setae probably microtrichs, with very long skeletal cones.

Discussion.

The skeletal cones (p. 59) of the microtrichs suggest an essential affinity with 'macrotrichs' (p. 59). There is perhaps no sharp distinction between the two. Some trichs, such as those on the thoracic appendages of *Chirocephalus*, seem to present an intermediate condition. However there are usually a number of microtrichs to one epidermal cell, whereas one cell never bears more than one macrotrich (Text-fig. 5); but cells with a single microtrich are found (*Asellus*), and there are apparent microtrichs as long, or as thick, as macrotrichs (*Chirocephalus*). Perhaps more diagnostic is the uniform distribution of microtrichs over the body surface as

contrasted with the more sparse and irregular distribution of macrotrichs.

The literature on the integument of Crustacea contains numerous references to, and figures of, structures which in their general distribution and size, strongly suggest modified microtrichs. The scales of *Porcellio* (Wahrberg, 1922-4; Herold, 1913), the numerous spines and 'retinacules' of *Rhizocephala* (Boschma, 1930, 1931) and of *Argulus* (Guberlet, 1928) all show essential resemblance to this system. Zimmer (1927, p. 720 and p. 628) figures them in the stomodaeum of *Asellus* and *Praunus*, and the regular system of scales which replace the corneal facets in the region between the two parts of the eye in the Mysid *Euchaetomera* (Zimmer, p. 625) may also be microtrichal. Indeed, wherever a system of exoskeletal processes is uniformly developed on all cell areas of a region it is probable that it represents a modified microtrich system. On this criterion it seems possible that even the complex and thickened biting surfaces of the mandibles of *Asellus* may have evolved from microtrichs.

The fact that the microtrichs of *Asellus* are better developed and most abundant towards the centre of the body and progressively reduced peripherally, and along the limbs, might suggest that they are proprioceptive in function. On the other hand, the trichs are orientated to respond to stimuli from the periphery inwards, which suggests that they are primarily exteroceptive: their poorer development peripherally may be balanced by an increased amplitude of movement of the parts, increasing the amount of external stimulation. This may explain why the trichs are shortened all over the body surface in an active form like *Gammarus*. In some cases the pore canals alone may be sufficiently sensitive. In aquatic forms the trichs are probably sensitive to any movement on the surface of the body, including water movements, and it seems possible that the increased surface of the trichs in the terrestrial Isopoda (Wahrberg, 1922-4) renders them sensitive to air currents.

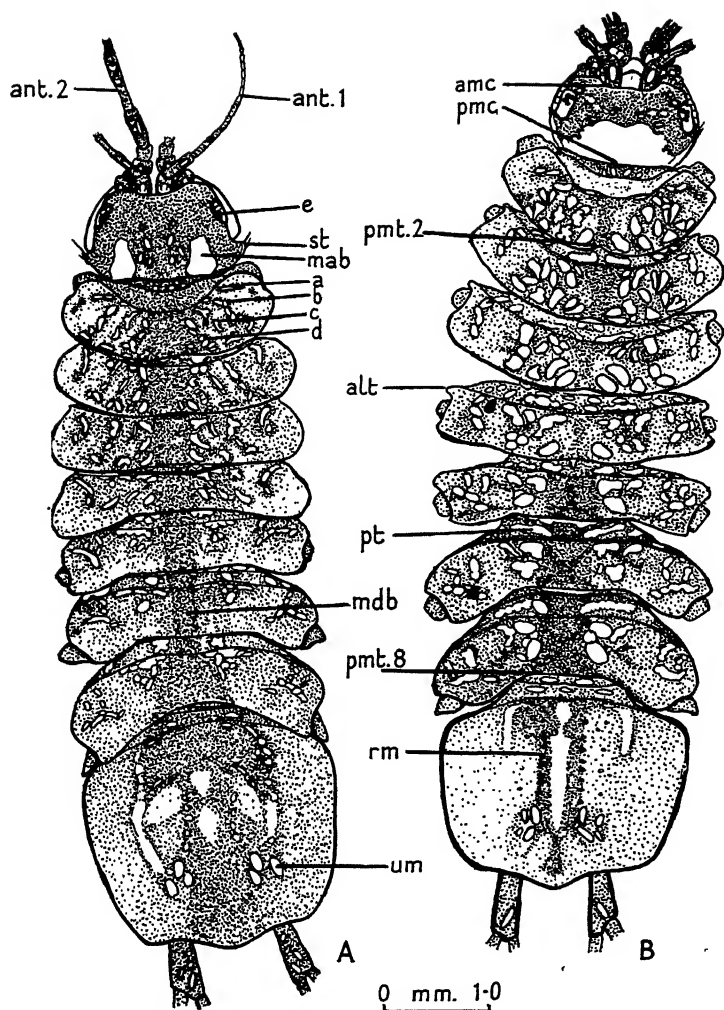
The special orientation of trichs on certain appendages (e.g. pleopods in *Asellus*) is no doubt correlated with specialized movements (Needham, 1938) and the specially long ones on the

mouth parts of *Asellus* and *Gammarus* and on the thoracic limbs of *Chirocephalus* have probably assumed an additional, mechanical function. Secondary functions are probably common (e.g. retinacula of *Rhizocephala*).

4. THE PIGMENTARY PATTERN IN ASELLUS.

The colour of *Asellus* is due chiefly to a melanin (Baldwin and Beatty, 1941), distributed in granular form in the cytoplasm of branched pigment cells underlying the epidermis, giving the appearance of a complete covering of pigment, under low powers of the microscope. The visible pattern on the dorsal surface (Text-fig. 7) is due to the absence of pigment cells from certain areas. Pigmentation is sparse on the ventral surface, except that of the head, and of the abdomen, which is exposed when the abdomen is raised in respiration. Other parts of the ventral surface develop pigment if exposed to direct light; after removal of the operculum pigment is visible on the underlying gills within 24 hours. On the appendages, again, pigment is better developed on the dorsal surface.

The pattern on the dorsal surface is characteristic (Text-fig. 7). The areas which break up the background of pigment are round or oval, or may coalesce to form larger areas of varying shape. The arrangement of these areas is constant, and is found to depend on the arrangement of the muscles of the body. Arthropod muscles originate between the epidermis cells on a broad area of the exoskeleton and from all these areas of attachment pigment cells are excluded. On a typical thoracic segment (e.g. thoracic 2, Text-fig. 7 A) the muscle impressions on each side are roughly arranged in four series of concentric arcs, their concavities facing laterally. The small coxal segment has muscles inserted on its lateral edges only, and it is the origin of these which is responsible for the small and rather separate lateral arc *a*. This is present in the second thoracic segment, in spite of the partial fusion of the coxa with the body in that segment. The coxo-basal joint has a single hinge, on its lateral aspect, and the basis musculature takes the form of a set anterior to this, rotating the limb forwards (anterior rotators), and a posterior set rotating it backwards



TEXT-FIG. 7.

Dorsal view of A. *Asellus aquaticus* male; B. *Asellus meridianus* male to show pigmentary pattern and other features.

(posterior rotators). Both sets fan out to their origin on the terga. The posterior rotators tend to pass medially to the

anterior rotators and so are responsible for the most medial arc *d*, while the anterior rotators form arcs *b* and *c*.

The pattern varies in the different segments of the thorax. A study of the small lateral group *a* (Text-fig. 7 B) gives the clue to this. In passing back it is seen to rotate progressively outwards and back (i.e. anti-clockwise on the left and clockwise on the right) through almost 180°. This follows a correlated change in the position of the coxo-basal hinge and in the orientation of the limb itself (Tschetwerikoff, 1910): both face obliquely forwards in anterior segments, and obliquely backwards posteriorly. The rotation is reflected in the shape of the terga also, but the rotation of the muscle origins is greater than that of the terga, so that in posterior segments many of them move forwards off the tergum on to the pretergal part (Text-fig. 7, *pt*). Further, some of the origins of the anterior rotators tend to rotate in the opposite direction, i.e. backwards, so that they remain in the same position relative to the tergum (segments 6, 7, 8 thoracic). The shape of the impressions, also, varies according to the obliquity of the muscles.

On the head the two large impressions *mab* are due to the mandibular abductor-levator muscles. On the abdomen the muscles of the uropods (*um*) are clear posteriorly, and the most anterior impressions are those of the pleopod muscles (Pls. 3-5). Of the other impressions on the abdomen the median longitudinal one is due to the close proximity of the hind gut and to its musculature *rm*, while the many small areas on either side are due to fibres traversing the abdomen dorso-ventrally.

The appendages show a pattern, similarly determined by muscle impressions, on their dorsal surface.

Discussion.

Tylor (1886) and McCook (1888) have attributed some share in the determination of colour pattern in animals to underlying structures (nerves, muscles, bones, &c.) though such determination is inadequate to explain the disruptive markings of most animals, which appear to bear no relation to any particular morphological structure (Cott, 1940, p. 152). It seems possible, nevertheless, that anatomically determined colour patterns

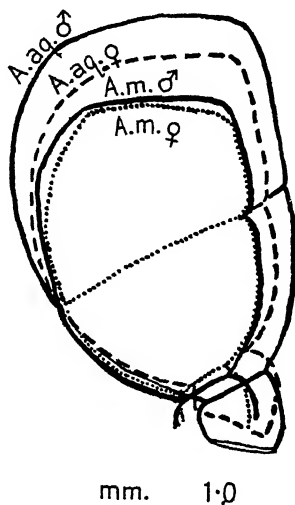
such as that of *Asellus* may have adaptive value, being inconspicuous in the normal environment. It is a very common (dappled) cryptic pattern. One must suppose that the details are unimportant and that a fortuitous pattern, determined by the musculature is sufficiently adaptive to persist unmodified. Certain elements which have been added to this pattern clearly indicate that the coloration of *Asellus* is adaptive. There is a darker band along the mid-dorsal line (*mdb*), a usual feature of cryptic pattern (Cott, 1940), counteracting the high light. There is also a definite lightening of tone towards the lateral margin, while the upstanding edges of the terga are darker. Thus the colour pattern of *Asellus* illustrates two important evolutionary tendencies, first the utilization of pre-existing systems (muscular system) for new purposes (colour pattern), and secondly the harmonious co-operation of different components in the determination of one character (total colour pattern); this latter is an example of the 'planing' action of natural selection (Needham, 1937, p. 311).

The pattern on the wood-loose *Porcellio* and the marine Isopod *Idothea* likewise depends on muscle impressions. In *Armadillidium* the slaty-coloured pigment typical of this wood-loose has encroached upon the muscle impressions, leaving only a few of them as white patches. Thus the pattern determined by muscle impressions may be partially obliterated: just as more than one component may be concerned in colour pattern so any particular component is not necessarily modified in its entirety. The condition in *Armadillidium* supports the view that the whole colour pattern is adaptive. On a muddy substratum the colour pattern of *Asellus* becomes obscured by fine silt which probably adheres to the microtrichs (p. 59) while in clear water the colour pattern is exposed.

5. FURTHER DIFFERENCES BETWEEN *ASELLUS AQUATICUS* AND *ASELLUS (PROASELLUS) MERIDIANUS*.

An account of these two species has been given by Racovitza (1919) and other points have been noted by Scourfield (1940). Further differences have been observed which are perhaps worth recording. *Asellus meridianus* differs from

Asellus aquaticus as follows: Colour: lighter tone, due to less crowded pigment cells; unpigmented areas larger (pigment cells encroach less at edges of muscle impressions). Pigmentary pattern (Text-fig. 7): limbs relatively shorter (Racovitza, l.c.), especially posteriorly, muscles shorter, so that muscle impressions do not spread so near mid-dorsal line; impressions do not



TEXT-FIG. 8.

Left operculum of both sexes of *Asellus aquaticus* and *Asellus meridianus* from individuals of the same body size.

move so far off terga in posterior segments; coxae more obvious in dorsal view (limb attachments carried more dorsally), muscle impressions therefore set nearer mid-dorsal line in anterior segments; impressions of mandibular abductor-levators consequently meet across mid-line (specific difference of use in the field (Scourfield, l.c.)); median dark band (p. 67) narrower anteriorly and wider posteriorly. Shallow median concavity in posterior margin of tergum of second thoracic segment (*pmt.* 2) narrowed to a mere notch, that of the eighth segment (*pmt.* 8), on the other hand, broadened. Postero-lateral setose tubercles (*st*) on cephalon minute whereas the antero-lateral tubercles of

the pleura of thoracic segments 5 and 6 relatively larger (correlated with more dorsal position of limbs). Differences in anterior and posterior margins of cephalon (*amc*, *pmc*). Operculum of respiratory pleopods smaller (Text-fig. 8), the difference largely in the distal segment, which also has relatively shorter median edge; operculum covers abdomen less completely. Oostegites of immature female (p. 57) relatively smaller, median edge pointed rather than square. Embryo: lateral lobes of characteristic trifoliate organ virtually absent. Physiological difference: less hardy and therefore less suitable for experimental work (? depends on relative size of gills (Text-fig. 8)).

SUMMARY.

1. The micro-anatomy of the autotomy mechanism in the thoracic appendages of *Asellus* is described; it shows considerable resemblance to that of *Astacus*, except in size.

2. The fifth thoracic pair of appendages do not autotomize in the mature male *Asellus*. This depends on a very small anatomical difference, the closure of a fissure which is present in the exoskeleton of normally autotomizing limbs.

3. The method of autotomy is inferred from observations, and from the arrangement of the muscles of the limb.

4. The micro-anatomical changes which accompany the very rapid expansion of the oostegites in the female *Asellus*, are described.

5. A system of 'microtrichia', probably homologous with that of insects, is described in *Asellus* and in certain other Crustacea. Its probable function is indicated.

6. The pattern of pigmentation in *Asellus* is largely determined by the muscular system of the body, but is, nevertheless, probably adaptive. The pattern in other Isopoda is similarly determined but may be modified.

7. Certain unrecorded differences between *Asellus aquaticus* and *Asellus* (*Proasellus*) *meridianus* are enumerated.

This work was done at Bedford College, London, and at the Physiology Department, University of Manchester.

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DESCRIPTION OF PLATE 2

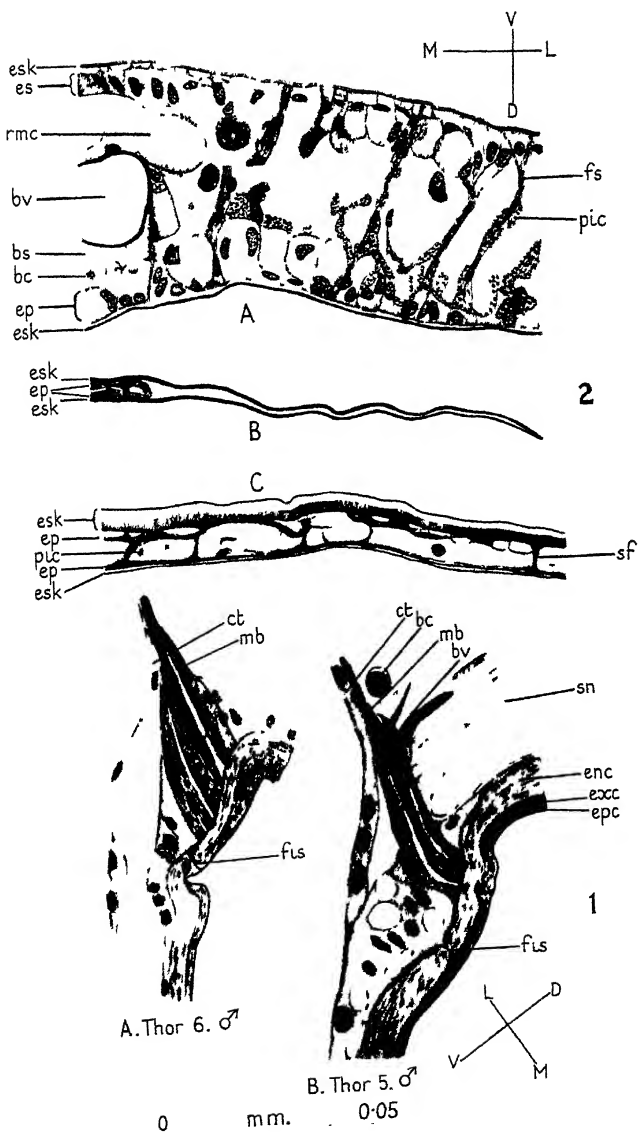
LIST OF ABBREVIATIONS OF PLATE AND TEXT-FIGURES.

a, b, c, d, four main arcs of muscle impressions; *alt*, antero-lateral tubercle of pleuron; *amc*, anterior margin of cephalon; *ant. 1*, antennule; *ant. 2*, antenna; *bc*, blood corpuscles; *bs*, blood sinus; *bv*, blood channel; *ca*, cell area; *cbj*, coxobasal joint; *cr*, crescent of microtrichs; *ct*, connective tissue covering autotomy membrane; *D*, dorsal; *e*, eye; *enc*, endocuticle; *ep*, epidermis; *epc*, epicuticle; *esk*, exoskeleton; *exc*, exocuticle; *fis*, autotomy fissure through exoskeleton; *fs*, fibrillar strands; *L*, lateral; *M*, medial; *m*, autotomy muscle; *ma*, macrotrich; *mb*, autotomy membrane; *mi*, microtrich; *pc*, pore canal; *pc. 1*, large pore canal on boundary of cell area; *pic*, pigment cell; *pmc*, posterior margin of cephalon; *pmt. 2, 8*, posterior margin of terga of thoracic segments 2 and 8; *pt*, pretergal macula; *rnc*, reserve material cell; *sc*, skeletal cone from base of trichs; *sn*, segmental nerve; *st*, setose tubercles; *V*, ventral.

PLATE 2.

Fig. 1.—Medial (ventral) part of longitudinal sections of autotomy region of thoracic appendages of adult male *Asellus aquaticus*: A, sixth thoracic (autotomizing). B, fifth thoracic (non-autotomizing). (Region enclosed by dotted line in Text-fig. 2.)

Fig. 2.—Parts of sections of oostegite of female *Asellus aquaticus*: A, transverse sections before expansion. B, C, longitudinal sections after expansion; B, near margin; C, near base. To show micro-anatomical changes which occur on expansion.



The Free Border of the Intestinal Epithelial Cell of Vertebrates.

By

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With Plates 3, 4, and 5, and 2 Text-figures.

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PREFACE.

THE chief or absorptive cells of the intestinal epithelium of Vertebrates present to the lumen of the gut a specialized border which has received many names. It has been called the free border, freie Oberfläche, Stäbchensaum, Stäbchenorgan, striated border, brush-border, Burstensaum, plateau, cuticle, cuticula, and Stäbchencuticula. In this paper the name 'free border' will be used, because it is self-explanatory and does not beg the question of the structure of the object named.

The structure of the free border is a matter of great physiological interest, because all the food that is absorbed must pass

through it. Although elaborate diagrams of the structure of the striated border are available, yet no accurate drawing, known to me, gives more than faint support to the details of those diagrams; and, so far as I am aware, no attempt has been made to reveal the structure by photographs. My own studies have made me believe that the currently held opinion as to the structure of the border is wrong. This paper is illustrated by photomicrographs. Although the photography of a membrane that is only about $2\frac{1}{2}\mu$ thick presents great technical difficulties, yet I have thought that nothing less than untouched photographs would suffice to make others doubt the accepted opinion.

In this paper the side of the free border next the lumen of the gut is called 'superficial', and the side where the border is attached to the cytoplasm of the cell is called 'basal'.

This is the first of a series of papers on the cytology of digestion. I wish to thank Professor E. S. Goodrich, F.R.S., in whose Department the work was carried out, for his advice and encouragement.

REVIEW OF THE LITERATURE.

Introduction.—The free border of the intestinal epithelial cells of Vertebrates was first figured by Henle in 1841. He called it the 'freie Oberfläche', and his drawing makes it appear structureless. He drew the cell membrane round the rest of the cell almost as thick as the free border, an error which must be ascribed to a diffraction-halo effect caused by the low numerical aperture of the objectives available a century ago.

Two years after Henle published his drawing the first paper appeared which suggested that the free border of the intestinal epithelial cell was not structureless. Since then there have been two main opinions as to what the structure is. All have agreed that striations are visible, running perpendicularly to the surface. One group of workers has thought that the striations indicate that the membrane is composed of cilia or of immobile rods resembling cilia, while the other has thought that the membrane is pierced by narrow cylindrical tubes or 'pores', which give the striated appearance. The histories of the two opinions may be related separately.

The Cilium or Rod Theory.—In 1843 Gruby and Delafond announced that there exist vibratile bodies at the surface of the epithelium of the small intestine of the dog. They thought that the function of the vibratile bodies was to displace the chyle where it is in contact with the epithelium. It must be regarded as rather likely that Gruby and Delafond saw the striations whose nature has given rise to so much discussion, but those subsequent workers who have upheld the cilium or rod theory have not regarded the cilia or rods of adult Vertebrates as mobile.

It will be recollected that the gall-bladder and larger bile-ducts have an epithelium closely resembling that of the small intestine, and it was here that Friedreich described what he called fused ('verklebten') cilia in a $3\frac{1}{2}$ months' cow embryo in 1857. Next year (Friedreich, 1858) he described the same organs in a child. He noticed the division of the membrane into superficial and basal layers, and he described the striation of the latter.

Zimmermann (1898) studied cells from the crypts of the colon of man. (The crypt cells are scarcely distinguishable from those of the intestinal villi.) He thought that the membrane consists of an inner cuticle with rods embedded in it (Stäbchencuticula), and of an outer part formed of fine 'Pseudopodia', each pseudopodium being a continuation of one of the embedded rods.

M. Heidenhain (1899), who was influenced by Zimmermann's work, fixed his attention on salamander larvae, and his paper has had a profound influence on opinion ever since. He described the membrane as composed of a basal and a superficial part, both striated. The basal part, he maintained, consists of strong rods, the Innenglieder, swollen at both ends. Superficially lie the thin Aussenglieder, which he described as fine protoplasmic filaments resembling cilia. He did not say that they were motile, but he claimed that the Innenglieder were 'etwas beweglich'. A count in one of his figures shows about 110 Aussenglieder to 53 or 54 Innenglieder. R. Heidenhain (1888) had already drawn a figure very similar to M. Heidenhain's many years before. In this figure there are about 40 striations corresponding to Aussenglieder to 21 or 22 bodies corresponding to Innenglieder.

M. Heidenhain said that sometimes only the Innenglieder are seen, which he attributed to faulty fixation.

It is to be remarked that in the larvae of many Amphibia part of the intestine is genuinely ciliated. This applies to the tadpoles of frogs and toads (Gage & Gage, 1890: Kaywin, 1936), and it does not seem impossible that the larvae of salamanders also have cilia on parts of their intestinal epithelium, though Gage & Gage say that ciliation is restricted in them to the oesophagus. (Salamanders are unfortunately not available in Britain during the war.) The cilia are lost from the intestine of tadpoles before metamorphosis, and the border left behind is striated like that of other Vertebrates.

The year 1899 saw the publication not only of M. Heidenhain's important paper, but also of another giving strong support to the cilium-theory. This was Prenant's comparison of 'cellules à plateau' with ciliated cells. Many years before, Frenzel (1886) had shown that the cilia of certain Invertebrate epithelia have basal granules. Prenant now described similar granules on the basal side of the striated border of the intestinal epithelial cell of Vertebrates, and these appeared to him strong evidence that the membrane consisted of cilia, which he thought were fused together by a cementing substance. It should be mentioned that Mall (see Zipkin, 1904) had seen a similar appearance long before. Prenant found that both the basal granules and the intra-cytoplasmic parts of the cilia stained with iron haematoxylin, while the superficial parts of the cilia did not, and there was thus a division of the border into basal and superficial layers. Prenant did not claim that one striation corresponds with one basal granule, and he admitted that the granules lying at the base of the free border are unlike those of a ciliated epithelium in being irregularly arranged, often angular, and sometimes crowded together so as to touch. Prenant (1911) repeated his comparison with ciliated epithelium a dozen years later.

M. Heidenhain's and Prenant's papers, appearing at almost the same time, had an influence which persists to the present day. The first notable adherent to their views was Zipkin (1904), who studied the intestinal epithelium of the Rhesus monkey.

She distinguished a basal and a superficial layer in the free border. Both layers she described as traversed by rods. In this she differed from Thanhoffer (1874), who had described and figured basal and superficial layers in the frog, but had confined the striations to the superficial part.

Zipkin thought that the parts of the rods (Innenglieder) in the basal layer were joined by a cementing substance, while the external parts (Aussenglieder) were free. At the base of each Innenglied she found a basal granule, and she thought she could see another granule in each rod at the line of demarcation between the basal and superficial layers of the border. She gave no accurate drawing of what she could see, but only a half-schematic one (her Fig. 4) and a wholly schematic one (her Text-fig. 15), which has been reproduced by Patzelt (1936) and in a modified form by Clara (1926). It is to be noted that Zipkin made the number of Aussenglieder correspond exactly with that of the Innenglieder and basal granules, though she herself says that the basal granules (or 'ellipsoids') were only in a few cases sufficiently distinct from one another to be counted. It is also to be remarked that she herself did not know whether the line of demarcation between the basal and superficial layers of the border was to be regarded simply as the sum of the outer granules. Her diagram, in fact, is a basis for discussion rather than a statement of what she could see.

Patzelt (1936) agreed in general with Zipkin, and claimed to have counted forty-one rods in surface-view of an intestinal epithelial cell of man. It is difficult to be certain that he was not counting only the basal granules. He noticed that the striations were visible in fresh cells. His own drawings give little support to the details of structure shown in Zipkin's diagram.

If Zipkin's diagram transcends the demonstrable, then Clara's modification of it (1926) transcends it still further. It has been shown (Baker, 1942) that his diagram includes details which are far beyond the limit of microscopical resolution. Clara worked with the intestinal epithelium of birds, and his drawings give no support to the details of his diagram.

The intestine of many Invertebrates is, of course, genuinely ciliated, with motile cilia. Each of these cilia generally has two

basal granules, a basal and a more superficial one. Newell and Baxter (1936) have made a careful study of the free border of the epithelial cells of the alimentary canal of Invertebrates. They find sometimes true ciliation, sometimes what they call a 'rod-border', and sometimes (in the mid-gut of Insects) a 'brush-border'. Neither in the rod-border nor in the brush-border are there basal granules, and the authors do not regard the rod or brush-elements as representing cilia. They do not identify the Vertebrate free border with any of the three kinds of border studied by them in Invertebrates.

The Pore Theory.—It cannot be regarded as certain that Gruby and Delafond (1843) actually saw the striations in the free border of the Vertebrate intestinal cell. If not, then the discovery is to be attributed to Funke. The first announcement was made orally by Kölliker on 7th July 1855, but Kölliker was the editor of the journal which published Funke's paper in September of the same year (Funke, 1855). Kölliker's published paper appeared in 1856. Funke described his surprise at finding what looked at first sight like resting cilia on the free border of the epithelial cells of the intestine of the rabbit. He realized that the free border was in fact striated, and he did not himself consider the striations as representing cilia. He remarked that they never showed movement. He thought that the striations (not the spaces between) might represent 'Porenkanälchen', through which perhaps fat-droplets might pass in absorption. He viewed the cells not only from the side, but also in surface-view, in which he thought he saw the Porenkanälchen as dots.

Kölliker (1856) studied the striations in mammals, a bird, and several Amphibia. He found the striations easiest to observe in the salamander and newt. Like Funke, he regarded the striations, and not the spaces between, as pore-canals, through which very minute fat droplets entered the cell. His figures are extraordinarily minute, so that the striations can scarcely be seen without a magnifying-glass.

The pore theory still found some acceptance a decade later, when it was affirmed by Leydig (1866) in the French edition of his histological text-book in these words: '... la cuticule [of the intestinal epithelium of Vertebrates] est traversée par des

canalicules poreux normaux à la surface ; ils donnent à la couche cuticulaire un aspect finement linéolé, et cette couche, vue par la surface, paraît être finement ponctuée.' From this time onwards, however, the pore-theory found little acceptance, and Heidenhain's and Prenant's papers of 1899 seemed almost to clinch the matter in favour of the cilium or rod theory. A partial exception is to be found in a drawing of Schneider's (1902), which represents the striated border in the frog. He drew rods held together by a cementing substance, but also drew rather a wide canal traversing the border parallel to the rods. He stated that such pore-like canals are not seldom found in the homogeneous cementing substance in the case of the frog.

During the forty years since Schneider published his drawing, little support has been found for the pore theory. M. Heidenhain himself, however, wrote somewhat equivocally on the subject in 1907. He said that a hard, stainable material sometimes occurs between the immobile bases of cilia, and that this material sometimes resembles a 'Porencuticula'. This appearance is repeated, he said, in the free border of the Vertebrate intestine. He considered the free border analogous to a ciliated border, but not necessarily homologous with it.

Comment.—My own studies have convinced me that the free border is traversed by canals perpendicular to its surface. There is a continuous substance between the crowded pores, and it is the optical section of this substance, lying between contiguous pores, which people have mistaken for rods or immobile cilia. I adhere, therefore, to the theory that the free border is traversed by pores, but I differ from those who upheld this theory in finding the pores much wider and of quite a different shape. Indeed, I believe (though of this I cannot be certain) that what they thought were pores were actually the optical sections of the substance lying between pores: in fact, the same appearance that the upholders of the other theory interpreted as rods.

The only true figure of a pore in my sense is that given by Schneider (1902), but he thought that these pores only occurred sporadically, and that rods held together by a cementing substance constituted the general structure. Schneider did not realize that all his 'rods' were optical sections of parts of a

continuous substance lying between innumerable pores. The single pore which he figured was not the unique object he thought it, but the basis of the whole structure of the border, which he misinterpreted elsewhere.

TECHNIQUE.

The small intestines of the following animals were used in this investigation:

Amphibia. *Triturus vulgaris* (smooth newt), adult and larval. *Triturus palustris* (crested newt), adult and larval. *Rana temporaria* (common frog), adult and larval.

Reptilia. *Anguis fragilis* (slow-worm).

Aves. *Sturnus vulgaris* (starling).

Mammalia. *Oryctolagus cuniculus* (rabbit).

Methods for Revealing Structure.

A wide variety of methods was tried, but only those which were found particularly helpful will be mentioned here. Reference to other methods will be found in the descriptive part of the text.

Fresh Preparations.—In the frog, careful teasing in 0.75 per cent. sodium chloride solution separates the cell sufficiently for the free border to be studied while the cell is still alive.

Maceration.—Goodrich's boric acid and iodine (1942) was used with success, as well as Ranvier's alcohol and other macerating agents. The intestinal cells of the newt are remarkably resistant to maceration.

Fixation.—When intestines provided with villi are to be sectioned, the orientation of the piece of tissue in relation to the microtome knife is of no significance, because anyhow the free border will be cut in diverse planes. The newts, however, have no villi. The small intestine is simply thrown into a few longitudinal folds. If a piece of intestine is fixed in such a way that it is held straight, one can make every section perfectly vertical through the free border by cutting sections transversely through the piece. This is a considerable advantage for some purposes. A piece of newt intestine (not slit longitudinally) is laid straight along a headless match-stick, and the latter floated on the fixa-

tive. When it is wished to get oblique and horizontal sections through the free border in the newts, the intestine is fixed in a tangled condition.

The smallest frog tadpoles were fixed whole, but the intestines of older specimens, in the opercular stage, were separated from the body before fixation.

When it was wished to preserve the free border with a fixative which does not precipitate proteins, formaldehyde was generally used. The fixing solution was made by diluting 2 c.c. of formalin with 18 c.c. of 0.75 per cent. or 0.9 per cent. sodium chloride solution.

The following fixative was generally used when the sections were going to be stained with safranine-acid-violet (see below):

Mercuric acetic sulphate (M.A.S.).

Mercuric chloride, sat. aqueous solution 98 c.c.

Acetic acid (glacial) 2 c.c.

Sodium sulphate 1 gm.

The sodium sulphate is included in this fixative for its osmotic effect. Sodium chloride is unsuitable for this purpose in fixatives containing mercuric chloride, because it forms a double salt with the latter. Fix overnight and wash in iodine-alcohol.

Helly's fluid was found to be a good fixative when mitochondrial techniques were used to show the granules of the basal layer of the free border. Fix for a few hours and then leave in a saturated aqueous solution of potassium dichromate at 40° C. for two days. (The saturation is done at room-temperature.)

For osmium impregnation fix for a day in Nasonov's modification of Champy's fluid, wash, and post-osmify in 1 per cent. osmium tetroxide solution at 30° C. for about a week (with newt material).

No other fixative tried gave results superior to those given by the four mentioned—formol-saline, M.A.S., Helly, and Nasonov. Of these the two first-named were much the most useful for most purposes.

Section cutting.—To reveal structure in such a very narrow membrane as the free border it is necessary to use very thin sections. Frozen sections are useful in revealing chemical

composition (see below), but only paraffin (preferably of melting point about 57° C.) will give thin enough sections for the work on structure. The most useful thickness is $2\ \mu$ or $3\ \mu$. It is easy to cut $2\ \mu$ sections of soft material with the excellent flat-cutting microtome made by the Cambridge Instrument Co., and provided for my use by the Christopher Welch Trustees. This instrument has the great advantage of maintaining an even thickness when it is set to cut very thin sections, instead of cutting alternately thick and thin (or thick and nothing!) as some microtomes do. To flatten $2\ \mu$ sections is not easy, but for cytological (as opposed to histological) purposes it does not matter if a considerable part of the section is folded, provided that other parts remain flat and not obscured by folds.

Staining.—One staining method stands out above all others for revealing the structure of the striated border. This is Bensley's (1911) safranin-acid-violet, which was used by him for quite a different purpose. The dye is a 'neutral' dye, of which acid violet is the acid radicle and safranin the basic. Acid violet, or Coomassie Fast Violet R (No. 698 in Rowe's 'Colour Index'), is obtainable from the British Drug Houses, Ltd. It is a complex triphenylmethane dye. Dissolve it at 4 per cent. in hot distilled water, cool the solution, and add an equal volume of 4 per cent. aqueous safranin O. A precipitate of the neutral dye begins to form instantly. When it is complete, wash the precipitate on filter paper with distilled water until the washing water is only pale pink from excess of uncombined safranin. Dry the precipitate and dissolve at 4 per cent. in boiling absolute alcohol by use of a reflux condenser. Cool the solution, mix with an equal volume of distilled water, and filter. The filtrate is the staining solution.

Stain sections fixed in M.A.S. for varying periods (less than an hour up to twelve hours), dry rapidly with filter paper, and dip quickly in this solution:

Absolute alcohol	.	.	1 vol.
Clove oil	.	.	3 vols.

Pass into xylene and examine under the microscope. Dip back again into the clove-oil-alcohol solution, repeatedly if

necessary, until the desired result is obtained; that is, when the safranin has been extracted so that it is confined or almost confined to the nuclei, while the acid violet colours the striated part of the border in blue or violet-blue more darkly than the cytoplasm. It is the affinity of acid violet for the 'canal layer' of the free border, which is difficult to stain differentially in any other way, that gives the method its special value in this study. It enables the free border and its parts to be recognized when sections would otherwise be difficult to interpret by reason of their obliquity. It is not important that the striated part of the border should be very deeply stained by the acid violet: it is only necessary that it should be so much more deeply stained than the cytoplasm that confusion is impossible.

The method does not work satisfactorily after most fixatives. M.A.S. is the fixative of choice.

Iron haematoxylin was used as a routine stain. It has no special affinity for the striated part of the border, but stains well the part which I call the granular layer.

I have found Benda's alizarin-crystal-violet the most suitable mitochondrial technique for revealing the granules of the granular layer. The granules stain with the crystal violet like mitochondria, while the canal layer is yellowish with alizarin.

A few other stains will be mentioned in the histochemical and descriptive parts of the text.

Mounting.—Stained sections were generally mounted in balsam, but I have done a large part of this work with unstained paraffin sections, and the structure could not otherwise, I think, have been fully revealed. The sections are brought down to water, covered with a coverglass, and subjected to critical examination under the microscope without any staining. When it is wished to keep the preparation permanently, 4 per cent. formaldehyde solution (10 c.c. of formalin to 90 c.c. of distilled water) is used instead of distilled water as the mounting medium. A round coverglass is used, which is cemented firmly to the slide with gold size applied by the aid of a paint-brush and a turntable. This method, which depends for its usefulness on the varying refractive indices of the structures studied instead of their capacity to retain stains, is one which I would strongly

recommend to cytologists as an addition to their ordinary techniques. Water (or weak formaldehyde solution) is chosen as mounting medium because of, not despite, its low refractive index. Indeed, I made experiments with methyl alcohol, because its refractive index is even lower; but the trouble involved in making a really efficient seal for methyl alcohol made me prefer weak formaldehyde solution as my standard method.

The superficial membrane of the free border stands out as in a diagram when this method is used. It is scarcely visible by ordinary methods and has escaped previous study.

Microscopy and Photomicrography.—A Watson 'H' Edinburgh Student's microscope was used, the very accurate fine adjustment being a great help. The optical equipment was generally a Watson $\times 14$ holoscopic eyepiece, a Reichert oil-immersion objective of N.A. 1.37, and a Watson parachromatic condenser of aplanatic N.A. 0.9. The objective and condenser were accurately centred not only to one another, but also to the eyepiece. This was achieved by the use of the Sloan objective-changer and Watson centring substage. Polarized light was obtained by the use of a sheet of 'Polaroid' in the substage ring. Another sheet in a special revolving mount above the eyepiece served as analyser. This polarizing apparatus was provided for me by the Christopher Welch Trustees.

A darkened room was used for critical observations. Glare was prevented by the use of a small source of light. This is particularly important when aqueous mounting fluids are used. The iris diaphragm of the condenser was always used as wide open as possible, and usually at the full aperture, so as to avoid errors caused by diffraction haloes.

Photographs were taken with the same microscope and equipment as that with which the visual work was done. The object having been carefully focused visually, a very small change of the tube-length sufficed to focus it on the plate. The fine adjustment was not touched, and the corrections of the objective were thus not disturbed. An Ilford 'Micro 3' screen was used for the unstained preparations and for some of the stained ones. The Chance Watson No. 4 filter was found valuable for the safranin-acid-violet preparations, as it strengthens the blue.

Ilford Rapid Process Panchromatic plates were used. The photographs were all taken at the magnification of 1210 diameters, and were subsequently enlarged to approximately 2500 diameters. The enlargement was done so as to exhibit the ultimate detail in the published plates. I want to thank Mr. P. A. Trotman for the care and skill which he has devoted to the process of enlargement. All the photographs are untouched.

Histochemical Methods.

Proteins.—For the detection of proteins, or rather of amino-acids containing a phenyl ring, tissue was fixed in 4 per cent. formaldehyde or in this simple protein precipitant:

Alcohol, 70 per cent.	.	95 c.c.
Acetic acid, glacial	.	5 c.c.

The tissue was washed and left for one or two days in 25 per cent. gelatine at 40° C. The gelatine was cooled and hardened with 4 per cent. formaldehyde solution. Sections were cut by the freezing microtome at 10 μ .

Sections were placed on a slide and the excess of water drained off. For the xanthoproteic test, drops of concentrated nitric acid were added. The sections were washed in water, and some of them were exposed to the fumes of ammonia. They were generally examined in water. Bensley's modification of Millon's reagent (Bensley and Gersh, 1933) was also used. The fluid was dropped on to the section and the slide left for some hours (at room temperature or in slight warmth) in a damp chamber. A coverglass was applied and the section examined while still lying in the reagent.

For the detection of nucleoproteins, tissue fixed in M.A.S. was treated by Feulgen's method (de Thomasi's modification (1936)). The sections were mounted in balsam.

Carbohydrates and related substances.—Baur's method (1933) for glycogen was used. The tissue was fixed in Benda's fluid, and the sections stained in Feulgen's reagent (as above) and mounted in balsam. To find whether any part of the membrane contained chitin, sections of tissue fixed in M.A.S. were brought down to water and tested with Schultze's chlor-

zinc-iodine, either with or without a previous overnight soaking in pure diaphanol (chloro-dioxyacetic acid) to remove any possible incrusting substances. The chlor-zinc-iodine was allowed to act for about two hours, and the sections were then washed in distilled water and mounted in 4 per cent. formaldehyde.

Fatty substances.—Lipines were tested for by the Smith-Dietrich test (Dietrich, 1910), with three modifications.

(1) A saturated aqueous haematein solution, acidified by the addition of 2 per cent. of glacial acetic acid, was substituted for Kultschitzky's haematoxylin.

(2) Weigert's borax and potassium ferricyanide differentiator was diluted with 40 times its volume of distilled water.

(3) Differentiation proceeded not simply 'overnight', but until the moment when the ground cytoplasm and chromatin had lost their blueness or greyness and become yellow.

Kaufmann and Lehmann (1926) and Lison (1936) regard a positive result with the Smith-Dietrich test as indicative of the presence of lipines.

The test is not convenient for very minute cytological work, because although one can cut frozen sections at less than 10 μ , yet the subsequent handling of them becomes very difficult when they have to be passed through a number of different fluids, some of them hot. For this reason I have introduced the following method, in which paraffin sections are used. It is rather long and complicated, but presents no technical difficulty.

Fix in Aoyama's fluid, a mixture of formaldehyde and cadmium chloride solution. This fluid was chosen because formaldehyde gives good general fixation without destroying lipines, while cadmium salts render them insoluble in certain solvents. After fixation for 24 hours dehydrate through grades of acetone, each grade containing cadmium nitrate. The final grade consists of:

Cadmium nitrate, sat. sol. in absolute alcohol	2 c.c.
Acetone	98 c.c.

Leave in this overnight at 40° C., to dissolve out any fatty or fat-like substances other than lipines. Bring down through descending grades of acetone, each containing cadmium nitrate,

and pass into 5 per cent. potassium dichromate at 40° C. Leave for two days, to render the lipines insoluble in all fat-solvents. Wash for seven hours in running water. The treatment so far described, except the actual fixation, follows the directions of Ciaccio (see Lison, 1936, pp. 206-8), but the remainder of the technique differs.

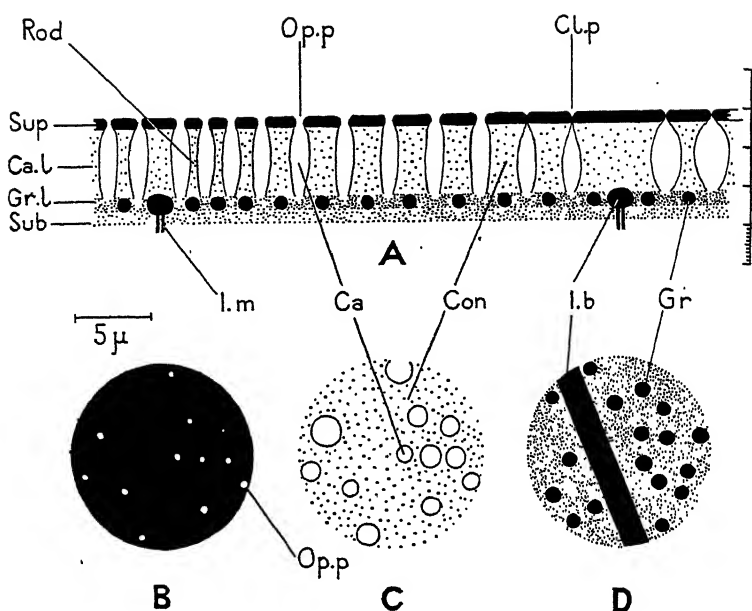
Embed in paraffin and cut sections at 3 μ . Bring down to water and leave overnight in saturated aqueous potassium dichromate solution at 40° C. Rinse with distilled water and leave for five hours in a saturated aqueous haematein solution, acidified by the addition of 2 per cent. of glacial acetic acid. Rinse. Differentiate in Weigert's borax and potassium ferricyanide, diluted with forty times its volume of distilled water, until the ground cytoplasm and chromatin have lost their blueness or greyness and become yellow. Wash in running water and either mount in Apathy's medium or carry through to balsam. This method of staining is a modification of the Smith-Dietrich method.

I believe that everything that is black or blue-black after this treatment is likely to contain lipines. Mitochondria are excellently shown in blue-black. I have suggested elsewhere (Baker, 1942) that, despite Bensley and Hoerr's (1934) conclusion, mitochondria may contain rather a high percentage of lipines.

RESULTS

The description which follows applies in general to the free border of the small intestine of the adults of all the species studied (see p. 80), but the details and measurements refer more particularly to the newts, especially the smooth newt, *Triturus vulgaris*. The chief differences found in the adults of the other species will be mentioned later, but it may be remarked that the differences concern the relative size of parts and not the general structure. The young frog tadpole shows a different and simpler structure (see p. 96).

Text-figures 1 and 2 are diagrams of what I believe to be the structure of the Vertebrate striated border. The figures are based on the newts, especially *Triturus vulgaris*. The photographs (Pls. 3, 4, and 5) illustrate the structure of the



TEXT-FIG. 1.

Diagrams illustrating the author's opinion as to the structure of the free border of the intestinal epithelial cell of Vertebrates. The proportions of the parts are based on the condition in the newts, especially *Triturus vulgaris*. The scale representing 5 μ applies to all four diagrams.

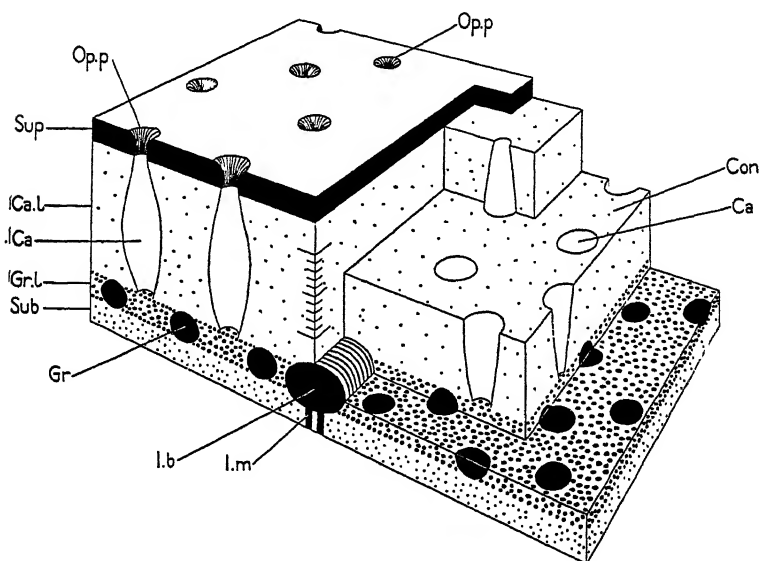
- A. Vertical section. (Compare especially figs. 3, 6, and 7 on Pls. 3 and 4.)
 B. Horizontal section through the superficial layer. C. Horizontal section through the canal layer. (Compare figs. 13 and 14 on Pls. 4 and 5.) D. Horizontal section through the granules layer. *Ca*, canal; *Ca.l*, canal layer; *Cl.p*, closed pore; *Con*, continuous substance of canal layer; *Gr*, granule; *Gr.l*, granular layer; *I.b*, intercellular band; *I.m*, intercellular membrane; *Op.p*, open pore; *Rod*, illusory rod-like appearance; *Sub*, sub-granular layer; *Sup*, superficial layer.

border in various species. Some animals show one part more clearly, some another.

The free border is divided into three layers: a thin superficial layer, a wide canal layer, and a basal layer containing many spherical granules. I have not been able to determine precisely

where the line of demarcation between the cytoplasm and the striated border should be drawn. The three layers will be considered separately.

The free border varies greatly in thickness according to



TEXT-FIG. 2.

Diagram drawn in perspective illustrating the author's opinion as to the structure of the free border of the intestinal epithelial cell of Vertebrates. The proportions of the parts are based on the condition in the newts, especially *Triturus vulgaris*. The scale represents 1μ divided into tenths. For explanation of the abbreviations see Text-fig. 1. The dots in this diagram and in Text-fig. 1 are intended only to distinguish the various layers. They do not represent granules. The walls of the pores and the surface of the intercellular band are also distinguished by conventional lines in this figure.

the degree of extension or contraction of the surface of the cell at the moment of fixation. The measurements given below and shown in the figure refer to the average condition seen in fixed preparations. When the cells are rather crowded together, the free border of each is somewhat convex distally. It is of the

same thickness over all parts of the cell, but gives the appearance of being pushed out slightly by the cytoplasm over the middle of the cell and held back at the margins where the cell is in contact with others.

When the microtome knife cuts at right angles to the plane of the free border, the section will be called vertical. Sections cut with the knife parallel to the plane of the border will be called horizontal.

The Superficial Layer.—This layer often scarcely appears in balsam preparations, a fact which accounts for its neglect. It shows very sharply in unstained sections mounted in water or 4 per cent. formaldehyde, as a dark line about $0.3\ \mu$ thick (fig. 2, Pl. 3). In stained preparations it is coloured, if at all, by basic dyes (e.g. methyl violet, pyronine) or basic dye-lakes (celestine blue and iron alum). It is sometimes blackened by fixation in osmium tetroxide solution (fig. 1, Pl. 3). It sometimes shows a trace of a positive reaction with Feulgen's reagent, which may indicate the presence of free aldehyde, as in plant cuticles. Its darkness in aqueous mounts interferes with the observation of colour-reactions in histochemical tests for proteins and chitin.

Critical observation under the highest powers shows that it is not uniform in width, but actual pores cannot usually be seen. When, however, the intestine of a newt is fixed in a tangled condition, so that some parts are stretched, pores become apparent (fig. 3, Pl. 3); and they can also be shown by pinning the intestine to a piece of cork with hedgehog spines in a stretched condition before placing it in the fixative (fig. 4, Pl. 3). (This method is not applicable to intestines provided with villi.) The superficial layer is now seen to be perforated with pores each about $0.2\ \mu$ in diameter. The difficulty in seeing these pores is caused partly by their minute size, which is small even in comparison with the depth of focus of the highest powers of the microscope, and partly by the fact that they are usually closed in fixed preparations. Fig. 8, Pl. 4 represents the superficial border of living intestinal cells of a frog, simply teased in saline and viewed in vertical optical section; and here the pores seem all to be open.

The dark appearance of the superficial layer is apparently not

due to any pigment, for it is so transparent as to disappear in horizontal sections. It is evidently of high refractive index, and it seems probable that, when it is viewed in vertical section in aqueous mounts, many rays of light are reflected from its surface in such a way as not to enter the microscope.

Below the superficial layer there is often seen (figs. 1 and 2, Pl. 3) a transparent, structureless layer of the same thickness ($0.3\ \mu$). This is not a simple diffraction effect, because it shows when the parachromatic condenser is used at full aperture. Indeed, I have seen it in an unstained preparation, using a Watson holoscopic oil-immersion condenser of N.A. 1.30 at full aperture, though the extreme transparency under these conditions made observation very difficult. Although this sub-superficial layer is not a diffraction effect, yet I regard it as an optical illusion, the converse of the dark rim seen round air-bubbles in microscopical preparations.

The Canal Layer.—This layer occupies the greater part of the thickness of the whole free border. It is about $1.8\ \mu$ thick. It consists of a continuous substance, and is traversed by canals placed at right angles to the plane of the border.

The continuous substance gives positive reactions to the xanthoproteic and Millon's tests, and therefore probably contains or consists of proteins. Tests for nucleoproteins, lipines, glycogen, and chitin give negative results. The fact that it is coloured green by the light green of Masson's trichrome stain suggests that it may contain collagen. Neither the canal layer nor any other part of the free border gives a positive reaction with Weigert's resorcin-fuchsin, and elastin may be taken to be absent. The canal layer has an affinity for acid rather than basic dyes or basic dye-lakes. The dye-lakes of haematoxylin do not stain it strongly. The most useful dye for it is acid violet, which colours it more strongly than the cytoplasm. This dye also has an affinity for collagen.

The canals perforating the canal layer (figs. 5, 6, Pl. 3; figs. 7, 8, 9, 10, Pl. 4) are spindle-shaped, attaining a diameter of about $0.5\ \mu$ at the middle of the layer. The diameter, however, is very variable. Each canal is continuous with a pore in the superficial layer (figs. 3, 4, Pl. 3; fig. 8, Pl. 4). The distance

from the centre of one canal to the centre of the next is very variable, but averages about $1.2\ \mu$.

The structure of the canal layer is most clearly seen when it is viewed in horizontal section (figs. 13 and 14, Pls. 4 and 5), for then the hollow canals and the continuous substance between are clearly seen. It would appear that the canals may sometimes communicate with one another. Parts of fig. 13, Pl. 4, for instance, suggest that this may be so.

The striations which give rise to the names striated border, rod-border, brush-border, &c., are only seen when the border is viewed in vertical section. They are optical sections of the continuous substance lying between the canals. In most species the canals are much closer together than in newts, and the amount of continuous substance is therefore much less. Optical transverse sections of the continuous substance give the illusion of rods standing up in a continuous surrounding space, but when one examines a horizontal section (e.g., fig. 13, Pl. 4 or fig. 14, Pl. 5) this appearance is at once seen to be false: canals are seen to perforate a continuous substance.

The special merit of the newt for studies of the free border resides in two separate facts. First, the absence of villi makes every transverse section through the intestine a vertical section through the free border. Secondly, the canals are further apart than in other forms, so that there is more of the continuous substance lying between one canal and the next, and therefore less chance that the intervening substance will give the illusion of being a 'rod'. Other species have their special advantages—the pores are wider, for instance, in the slow-worm—but the newt has the most diagrammatic arrangement. It has one disadvantage: the absence of villi combined with the presence of longitudinal intestinal ridges makes it much harder to get horizontal sections. Nevertheless, when one does obtain them (fig. 13, Pl. 4), they are exceptionally clear.

In all cases in which I have seen the canals in horizontal sections of the free border in various species, they have been arranged haphazard and not in regular rows. Their section tends to be circular, but some of them are often deformed (fig. 14, Pl. 5).

There is no possibility that the canals are artefacts. Not only do they appear in the same form whether a precipitant or non-precipitant fixative is used, but also they are clearly seen in living cells (fig. 8, Pl. 4).

Many attempts were made to get positive views of the canals, but only two methods succeeded. In Nasonov preparations intended to show the Golgi complex, the canals sometimes show as black spindles (fig. 11, Pl. 4). The facility with which osmium tetroxide is reduced on surfaces is probably the cause of this. The canals give no positive figure with any dye, acid or basic, nor any reaction with histochemical tests for proteins, nucleoproteins, lipines, or glycogen. They sometimes appear sporadically as greyish spindles when viewed in unstained, aqueous preparations. This must be due, I think, to the reflection of light from the surfaces of the canal in such a way that it does not enter the microscope. Treatment with $\frac{1}{2}$ per cent. iodine in 70 per cent. alcohol sometimes makes the canals appear dark (fig. 12, Pl. 4). I have found it best to treat the sections for some hours with this fluid, and then apply a coverglass without changing it. The canals look grey or blue-black, but no histochemical conclusion can be drawn, I think, from this appearance. Oblique and horizontal sections suggest that the iodine is often confined to the walls or ends of the canal, as though the iodine were adsorbed on the surface.

When cells are macerated in iodized serum and examined between crossed polaroids, a certain amount of light comes from the canal layer. It may be suggested that light is polarized by being reflected at the surfaces of the canals.

The Granular Layer.—Below the striated layer comes a layer which is very easily demonstrated, because it often shows in routine preparations stained with iron haematoxylin (fig. 17, Pl. 5). It generally appears in vertical sections as a stainable band about 0.4μ thick. It appears grey in unstained preparations (fig. 2, Pl. 3), and dark grey or black in unstained material fixed in osmium tetroxide (fig. 1, Pl. 3). In mitochondrial preparations it takes the same colour as the mitochondria (violet with Benda's technique).

This layer gives no positive reaction to tests for proteins or

glycogen, but it is positive for lipines by both the techniques described above (p. 86).

The layer contains spherical granules about $0.4\ \mu$ in diameter. They can often be seen as grey dots when paraffin sections of intestine fixed with mercuric-acetic-sulphate are mounted unstained in 4 per cent. formaldehyde. They become more definite and blacker when the sections are treated for an hour or two with Schultze's chlor-zinc-iodine before being washed and mounted in the same fluid. They become more definite still if the sections (preferably only $2\ \mu$ thick) are left in diaphanol (dioxychloroacetic acid) until the striated part of the border has dissolved away, then washed in running water and treated as before with chlor-zinc-iodine and mounted in formaldehyde solution. The granules are more distinctly separate when treated by this method than by any other (fig. 16, Pl. 5), though their separateness is sometimes evident in mitochondrial preparations (fig. 15, Pl. 5). The granules can be seen in unstained cells macerated in iodized serum. Between crossed polaroids they appear as dark spheres lying in a rather brightly illuminated layer.

It is questionable whether it is the granules alone which give the staining and histochemical reactions of the layer, or whether there is a similar but diffuse substance lying between them, which is cleared away by the diaphanol.

It was these granules which Prenant saw, and which he compared with the basal granules of cilia. He recognized that they are less obviously separate than true basal granules (p. 76), and I am inclined to associate this with the presence of a diffuse substance lying between.

So far as I can see, the granules do not lie directly below the canals of the canal layer, but alternate with them. This is suggested, e.g., by figs. 3, 4, Pl. 3 and fig. 8, Pl. 4, but it is not a matter on which I should like to make a dogmatic statement. They do not appear to be arranged in any regular way. Their significance is unknown.

When authors have claimed to see the rods as dots in surface-views of the stained cell, they have probably seen only the granules of the granular layer.

Below the granule layer there often appears a subgranular layer about $0.3\ \mu$ thick. This is shown particularly clearly in fig. 17, Pl. 5. This layer sometimes stains more intensely with iron haematoxylin and other dyes than the ground cytoplasm below, from which it then seems sharply differentiated; but it is not always seen. I have no means of determining whether the line between the ordinary cytoplasm and this subgranular layer represents the limit of the cell itself. If so, the subgranular layer is to be regarded as a cuticular structure. It seems just as probable that this layer and also the granular layer are really intra-cytoplasmic.

Below the subgranular layer there is a layer of cytoplasm which is sharply marked off from the cytoplasm further below by being clear and devoid of mitochondria. This clear layer shows especially well in fig. 8, Pl. 4. (The subgranular layer is not seen, as the cell is living and unstained.) It appears also in figs. 3 and 6, Pl. 3.

The Intercellular Band or Kittleiste.—This is a cord which runs round between each cell and the next, at the level of the granular layer. It is seen in surface-view in fig. 19, Pl. 5 and in transverse section in fig. 18, Pl. 5. It changes its shape, as seen in transverse section, according to whether the cells are pulled apart or pushed together. In fig. 18, Pl. 5 the cells are crowded together and it is higher than wide; but usually it is wider than high. It is larger in section than the granules of the granular layer and thus extends a little into the subgranular and canal layers. The intercellular band shows by routine methods in the rabbit, but is best demonstrated by mitochondrial techniques in the newts. It is coloured violet, like the mitochondria and granules, by Benda's method, and gives a positive reaction to the test for lipines described on pp. 86-7.

Opposite the intercellular band the canal layer is sometimes split, as though the free border were secreted separately by the separate cells; but it is much more usual for it to appear to be continuous.

- A Comparison of the Free Borders shown by the Species Studied.—The description given above applies more particularly to the newts, adult and larval. Even in a larva of

Triturus vulgaris only 10 mm. long (including tail) and lacking hind limbs, the adult structure is already shown (fig. 5, Pl. 3).

In the frog the canals are much closer together than in the newt, so that in section (true or optical) the continuous substance between them gives the illusion of being a series of parallel rods, swollen at each end (fig. 8, Pl. 4). In frog-tadpoles about three weeks after emerging from the jelly, when hind limbs have not yet appeared, the adult structure is already shown in parts of the intestine, but in other parts there is only a very thin and apparently structureless cuticle, resembling the striated layer in staining blue with acid violet. In tadpoles with external gills and no limbs, on the third day after emerging from the jelly, the adult structure of the striated border is nowhere seen. In places there is an excessively thin structureless cuticle staining with acid violet.

In the slow-worm the canals of the striated layer are wider than in any of the other species studied, so that horizontal sections are very striking (fig. 14, Pl. 5). They are crowded closer together than in the newt, and the smaller amount of intervening substance makes vertical sections more difficult to interpret.

In the starling the widest part of the canals is towards the bottom of the canal layer, instead of in the middle of it (fig. 9, Pl. 4).

In the rabbit the canals are closer and straighter-sided than in the newt. They are seldom so definitely spindle-shaped as in fig. 10, Pl. 4. The granular and subgranular layers and also the intercellular band are easier to show than in the other species (fig. 18, Pl. 5), but the separateness of the granules is not so evident as in the newt.

DISCUSSION.

The pore theory grew up round the idea that fats were absorbed by the epithelial cells of the intestine as minute globules. The upholders of the theory thought that such globules entered the cells through the pores. When the hydrolysis of fats was fully understood and the rod theory had gained

currency, both the pore theory and the physiological need for it lapsed.

Frazer, Stewart, and Schulman (1942) have recently shown that fats are only partly hydrolysed by the pancreatic juice. Fatty acids pass by the portal veins to the liver, unhydrolysed fat into lymphatics and thus to fat depots. These authors have shown that olive oil gives an extremely fine emulsion when mixed with oleic acid, cholesterol, and sodium hydroxide solution. They think that droplets of $\frac{1}{2} \mu$ diameter can be ingested through the free border. They showed that even paraffin is ingested if emulsified with oleic acid and cholesterol.

The relatively large canals which I have demonstrated in the free border seem to provide just the sort of structural basis that the physiological findings of Frazer, Stewart, and Schulman require. If the fat globules were a little smaller than the $\frac{1}{2} \mu$ mentioned by these authors, there seems to be no reason why they should not pass into the cell. The hypothesis that fat may be absorbed as such gains weight from the fact that two entirely different and independent lines of investigation point in the same direction. It now remains to demonstrate the fat actually passing through the canals.

In fixed tissue the pores usually, but not always (figs. 3 and 4, Pl. 3), seem to be closed or nearly closed, though in life they can sometimes be seen to be open (fig. 8, Pl. 4). It is possible that they open and shut as the cells are pulled apart or pushed together during movements of the intestine or villi. Indeed, this may be one of the reasons for the movements of the villi and an explanation of their musculature. In the newts, which have no villi, the longitudinal ridges of the intestine may play the same part. The free border is markedly elastic, being sometimes very thick (fig. 1, Pl. 3), and sometimes very thin (fig. 3, Pl. 3). Its elasticity has been practically demonstrated by Thanhoffer (1931). When lateral tension on the cells is relaxed, the pores may be supposed to close by the elasticity of the border. On further relaxation there would be a tendency for anything that had entered the pore to be pushed by the walls of the canal into the interior of the cell.

An alternative possibility suggests itself as to the mechanism

of the entry of substances from the cavity of the intestine into the epithelial cells. No simple explanation of the uptake of water in terms of osmosis is possible, since it occurs even from hypertonic solutions; but the canals provide a hypothetical explanation in terms of electro-endosmosis. It is very unlikely that the iso-electric point of the proteins of the canal layer is the same as the pH of the intestinal juice in the canals. The proteins, then, must carry an electric charge. If so, the canals provide a mechanism for the flow of water by electro-endosmosis. The direction of the flow would depend on the electric charge at the inner end of the canals, that is, at the granular layer. The function of the granular layer may be to maintain the necessary directive electric charge. If an actual current of water passes through the canals, it will carry amino-acids, mono-saccharides, salts, and other dissolved substances with it, and very finely emulsified fats might also be swept along.

It is not possible to demonstrate that the canals penetrate the granular layer, and it is here, therefore, that anything absorbed through the canals must be regarded as entering the substance of the cell. This must be the situation, then, where the distinction is made between those substances which can be absorbed and those which cannot. Very little is known about the processes which occur at this situation, but there are some indications of those connected with the absorption of glucose. The exact localization of phosphatase in the intestinal epithelial cell has not been determined, but the work of Gomori (1941) and of Kabat and Furth (1941) suggests that this enzyme, which is connected with glucose-absorption, may be particularly concentrated in the vicinity of the granular layer.

The free border is a very difficult object to photograph. It is presumably for this reason that no photograph has previously been published, so far as I know, with the intention of illustrating its structure. The photographs accompanying this paper will probably leave some readers unconvinced, though the actual preparations from which they were made would, I think, convince them. I would ask anyone who is sceptical to make preparations for himself, beginning with the newt (either of the two species mentioned). One straight and one tangled piece of

intestine should be fixed in M.A.S. (p. 81). Sections should be cut at $3\ \mu$ or $2\ \mu$, stained in Bensley's safranine-acid-violet (p. 82), mounted in balsam, and examined under the highest useful powers of the microscope with critical illumination. I believe that anyone who does this is likely to agree with me as to the structure of the canal layer, which is the crux of the whole problem. In the newt the wide separation of the canals makes the interpretation of the structure easy. Anyone who has seen the structure in the newt is likely to be able to recognize it again in other forms, in which the closer crowding of the canals gives the optical illusion, in transverse section, of the existence of separate 'rods'. Those who go on to try aqueous mounts will have no difficulty in seeing the superficial layer, though the presence of pores in it will not be confirmed without painstaking study. The granular layer has long been known. It shows well by mitochondrial techniques and even, in some animals, in routine preparations. The granules themselves, more or less separate, have been seen by various authors, but their resolution is not very easy.

SUMMARY.

1. The structure of the free border of the epithelial cells of the intestine has been investigated in various adult and larval Amphibia and in a reptile, bird, and mammal.
2. The structure is essentially the same in all (except in frog-tadpoles in the stage with external gills). This structure is shown diagrammatically in Text-figs. 1 and 2.
3. The free border consists essentially of three layers:
 - a. A superficial layer, pierced by pores, next the lumen of the intestine.
 - b. A canal layer, pierced by spindle-shaped canals continuous with the pores of the superficial layer. These canals traverse the layer at right angles to its surface.
 - c. A granular layer, containing many spherical granules, next the cytoplasm of the cell.
4. The intercellular band or Kittleiste is situated at the level the granular layer.
5. The continuous substance of the canal layer—that is, the

substance that intervenes between the canals—appears to be composed of proteins. The granular layer contains lipines.

6. The theory that the free border contains 'rods' is found to be erroneous.

7. It is suggested that unhydrolysed fats may enter the intestinal epithelial cell through the pores and canals in the form of extremely fine droplets.

8. Two hypotheses are put forward, the one mechanical and the other electro-endosmotic, to account for the passage of substances from the cavity of the intestine into the epithelial cells.

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The titles of many of the papers have been shortened.

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DESCRIPTION OF PLATES 3, 4, and 5.

All the photographs are of the free border of the intestinal epithelium of Vertebrates, and all are vertical sections except where the contrary is stated. The preparations were mounted in Canada balsam except where other mounting media are mentioned.

All the photographs were taken at the magnification of 1,210 diameters and subsequently enlarged to approximately 2,500 diameters. A scale of $5\ \mu$ is shown on each Plate.

PLATE 3.

Fig. 1.—*Triturus vulgaris*. Osmium-saline, unstained, aqueous mount. The photograph shows the superficial and granular layers, and also the illusory 'sub-superficial' layer. (Photomicrograph Ref. 211.)

Fig. 2.—*Triturus vulgaris*. M.A.S., unstained, aqueous mount. The photograph shows the superficial layer and also the illusory 'sub-superficial' layer. (Ref. 237.)

Fig. 3.—*Triturus vulgaris*. M.A.S., unstained, aqueous mount. The pores in the superficial layer are clearly seen. (Ref. 254.)

Fig. 4.—*Triturus palustris*. Intestine fixed in stretched condition. M.A.S., unstained, aqueous mount. The dark mark in the canal layer to the left of the middle of the photograph is a flaw in the negative. Two pores in the superficial layer are clearly seen just to the left of it. Other pores are seen elsewhere. (Ref. 282.)

Fig. 5.—*Triturus vulgaris*, larva 10 mm. long. Ciaccio's fluid, postchromed, iron haematoxylin. The photograph shows the canals. (Ref. 199.)

Fig. 6.—*Triturus vulgaris*. M.A.S., celestine blue, aqueous mount. The photograph shows the superficial and granular layers and the canals. (Ref. 256.)

PLATE 4.

Fig. 7.—*Triturus palustris*, larva weighing 0.6 gm. M.A.S., safranine-acid-violet. The photograph shows the canals. (Ref. 274.)

Fig. 8.—*Rana temporaria*. Living cells teased in 0.75 per cent. sodium chloride solution, unstained. Vertical optical section. The photograph shows the pores, canals, and granular layer in a living preparation. (Ref. 248.)

Fig. 9.—*Sturnus vulgaris*. M.A.S., safranine-acid-violet. The photograph shows the canals. (Ref. 250.)

Fig. 10.—*Oryctolagus cuniculus*. Susa, safranine-acid-violet. The photograph shows the canals. (Ref. 280.)

Fig. 11.—*Triturus vulgaris*. Nasonov, post-osmified, unstained. The canals are seen as dark spindles. (Ref. 220.)

Fig. 12.—*Triturus palustris*. M.A.S., iodine-alcohol (mounted in ditto). The canals are seen as dark spindles. (Ref. 259.)

Fig. 13.—*Triturus palustris*, larva weighing 0.6 gm. Horizontal section. M.A.S., safranine-acid-violet. The canals are seen cut across horizontally. (Ref. 193.)

PLATE 5.

Fig. 14.—*Anguis fragilis*. Horizontal section. Formol-saline, safranine-acid-violet. The large canals are seen cut across horizontally. (Ref. 271.)

Fig. 15.—*Triturus vulgaris*. Helly, postchromed, Benda's alizarin and crystal violet. The photograph shows the granules of the granular layer. (Ref. 209.)

Fig. 16.—*Triturus palustris*. M.A.S., diaphanol, chlor-zinc-iodine, aqueous mount. The arrow shows the place where the separateness of the granules is most clearly seen. (Ref. 285.)

Fig. 17.—*Oryctolagus cuniculus*. Susa, iron haematoxylin. The photograph shows the granular and sub-granular layers and the intercellular band. (Ref. 228.)

Fig. 18.—*Oryctolagus cuniculus*. Susa, iron haematoxylin. The photograph shows the intercellular band in vertical section of the free border. (Ref. 231.)

Fig. 19.—*Oryctolagus cuniculus*. Horizontal section. Susa, iron haematoxylin. The photograph shows the intercellular band in horizontal section of the free border. (Ref. 227.)



1



2



3



4



5



6

5 μ



5 μ



On the Head of the Liopelmid Frog, *Ascaphus truei*.

I. The Chondrocranium, Jaws, Arches, and Muscles of a partly-grown Larva.¹

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With Plates 6 to 14 and Text-figures 1 to 7.

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¹ This paper was written in the spring of 1941 and has not been altered since.

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1. FORMER WORK.

Noble (1931, p. 486) has published a classification of the order Anura in which he places the primitive North American frog, *Ascaphus truei*, Stejneger, with the New Zealand genus *Liopelma*, in a separate family, 'Liopelmidae', which

lies at the base of his evolutionary tree. He thus believes that the Liopelmidae show more primitive characters than any other living family of frogs. With this view the writer is in full agreement, for much new evidence is given below of the primitive nature of the larval skull, jaws, gill arches, and muscles of the *Ascaphus* tadpole. De Villiers (1934) has given an account of various other attempts to classify this animal; he also is in agreement with Noble's classification.

The adult skull of *Ascaphus* was described by de Villiers (1934) and by Wagner (1934) from sections of one individual. These papers are mentioned in various places by de Beer (1937) and by me (Pusey, 1938), where reconstructions are given from their published figures together with criticisms of certain of their findings. Noble (1931) had already figured a palatal view of the adult bony skull (his fig. 81 *a*) and had given some account of its structure. The larval skull has never been the subject of detailed work, so far as the writer is aware, although Noble (1927, fig. 9) has published figures of isolated sections of larvae to illustrate particular points. Valuable accounts, however, of the habits of both the larvae and the adults are given by Gaige (1920) and Noble (1927). Gaige states that Van Denburgh (1912) has given some account of the (? adult) skeleton, but I have not had access to this paper.

Both de Beer (1937) and Pusey (1938) have stressed the importance of a knowledge of the structure and development of the skull of *Ascaphus* for the interpretation of the structure and evolution of the anuran skull in general. I hope that this will be the first of a series of papers aimed at filling this important gap in our knowledge of Anuran evolution.

2. MATERIAL AND METHODS.

The specimens of *Ascaphus* in my possession were collected from Dick Creek, in the Carbon River valley of the Rainer National Park, Pierce County, Washington State, U.S.A. The work is based on a single series of transverse sections of a partly-grown larva of *Ascaphus truei*. The overall length of the

animal was 28 mm.; the length of the tail, measured ventrally, was 17 mm. and measured dorsally, 19 mm.; there were no visible hind legs. By comparison with the size ratios of *Rana temporaria* and *Discoglossus pictus* tadpoles and with metamorphic stages of other *Ascaphus* tadpoles, it is probable that this specimen had completed from one-half to two-thirds of its larval life. It was, therefore, a well-formed larva with all its structures completely laid down; very little young cartilage is present in the sections.

The gut and the lenses of the eyes were removed and the specimen was stained in bulk with borax carmine. It was impregnated with 2 per cent. celloidin and was then embedded in wax and cut, as transverse sections, at 15μ . The sections were counterstained with picro-nigrosin. Drawings were made from every third section with a microprojector, and from them the various reconstructions were made, either by the 'contour' or the 'projection' method described in another paper (Pusey, 1939); these are graphical methods. Selected sections were also drawn with the aid of the microprojector. Towards the end of the work two additional tadpoles of *Ascaphus* were cut as transverse sections, and one post-metamorphic animal was similarly sectioned.

For comparison with the *Ascaphus* material, I used previously prepared sections of various stages of *Discoglossus pictus*, *Bombina variegata*, *Rana temporaria*, and *Salamandra maculosa*. I also had access to further sections of *Rana temporaria*, *Bufo vulgaris*, and various *Urodeles*, which are in the departmental collection of the Oxford University Department of Zoology and Comparative Anatomy.

3. ACKNOWLEDGEMENTS.

I wish to repeat my sincere thanks to Prof. James R. Slater and to Mr. John W. Slipp, of the College of Puget Sound, Washington, for the time and effort which they gave to collecting the material of *Ascaphus* and for their generosity in sending it to me; to Prof. Carl L. Hubbs and to Mrs. Helen T. Gaige, of the University of Michigan, who helped me in my early efforts to obtain material and sent me an adult specimen

of *Ascaphus*; to Mr. R. Maxwell Savage, for his generosity in supplying me with a wealth of young larvae of *Discoglossus pictus* and *Bombina variegata*; to Drs. E. S. Russell and C. H. Waddington, for their gifts of *Discoglossus* tadpoles; to Dr. Nellie F. Patterson, of the University of Witwatersrand, who made and sent to me a wax-plate model of part of the chondrocranium of *Xenopus laevis*; and to Prof. D. M. S. Watson, F.R.S., for welcoming me back to his Department to use the microprojector there, and for giving me much valuable information from his own researches. The work, as a whole, was carried out in Oxford, at the Department of Zoology and Comparative Anatomy. I therefore wish to end by thanking Prof. E. S. Goodrich, F.R.S., for his interest in my work and for reading the manuscript for publication.

4. A DESCRIPTION OF THE CRANIAL ANATOMY OF A SINGLE PARTLY-GROWN LARVA OF *ASCAPHUS TRUEI*.

(a) The Anterior End of the Cranium.

The cranial cavity is not shut off by cartilage anteriorly, but opens freely forwards (*oec*, figs. 15 and 16, Pl. 11; and fig. 18, Pl. 12). In this region, the side wall of the brain-case is pierced by the foramina for the olfactory nerves (*fol*, fig. 1, Pl. 6, and fig. 19, Pl. 13); these foramina open laterally and not forwards as in other Anurans and are at the anterior end of the skull, with very little cartilage in front of them. The trabecular horns in *Rana* and the modern-type frogs project from the floor of the skull a long way forwards into the snout, in front of the olfactory foramina. In *Ascaphus*, however, the cartilage which is equivalent to the horns of the other frogs, hangs down from the trabecular region of the front of the skull as a vertical flange placed below and largely behind the olfactory foramen (*ct*, figs. 1 and 3, Pl. 6; fig. 15, Pl. 11; fig. 19, Pl. 13). The lower edge of this flange is indistinguishably fused to the upper edge of the medial part of the supra-rostral system (*clsm*); there is therefore no exaggerated anterior development of the horn region of the trabecula such as is typical of other frogs. In

the absence of evidence from younger larvae, the actual limits of the horns, as opposed to the supra-rostral cartilage, must for the present remain in doubt. In passing forwards from behind, the floor of the cranium becomes thicker. In the thickness of the cartilage a pit is excavated from in front, ending blindly behind; this is well shown as *ap*, in fig. 16, Pl. 11; fig. 17, Pl. 12; fig. 19, Pl. 13. It is also shown in fig. 2, Pl. 6, where the floor of the pit appears as a cartilage bridge, *cb*, joining the upper edges of the median part of the supra-rostral system; the typical floor of the cranium makes the roof of the pit.

The nasal-sac lies laterally to the olfactory foramen and somewhat behind it and not in front of it as in other frogs; it is partially roofed in by a projecting ledge from the orbital cartilage and is supported below on a similar ledge, *cl*, from the region where the trabecular horn passes into the supra-rostral.

(b) The Supra-rostral System.

This system is figured from in front in fig. 16, Pl. 11, and from behind in fig. 18, Pl. 12; it is also seen in the various reconstructions, fig. 15, Pl. 11; fig. 17, Pl. 12; and fig. 19, Pl. 13; and in section in figs. 2, 3, Pl. 6; and fig. 4, Pl. 7. It consists of a single, median part (*clsm*) fused to the trabecular flanges (*ct*), and a pair of separate, lateral wings (*clsl*). The median part is a flat plate facing ventrally, with the typical V-shaped notch (*nsr*) cut out of its anterior edge, as in other larval frogs (fig. 17, Pl. 12). On its dorsal surface there are flanges rising up on each side of the middle line which are fused to the trabecular flanges mentioned above (fig. 3, Pl. 6; fig. 18, Pl. 12). Thus, with the assistance of the cranial floor, this medial piece encloses a tunnel, open in front and behind, which houses a preoral buccal cavity (*pm*, fig. 3, Pl. 6; fig. 4, Pl. 7). The V-shaped notch is continued backwards on the dorsal surface of the ventral plate as a groove, which thus probably indicates that this median structure is really of paired origin in development (see fig. 18, Pl. 12).

The lateral wings of the supra-rostral system are independent structures. They articulate with the lateral edges of the median piece (fig. 3, Pl. 6) and are loosely held to it, and to the epithelial

roof of the sucker in front, by ligaments (figs. 2, 3, Pl. 6). The free edges, both of the lateral pieces and of the median piece, are mutually sheathed behind in dense mesenchyme of the nature of procartilage (*dm*, fig. 4, Pl. 7). This mesenchyme is underlain by very deep epidermis, whose outer part is hardened into the horny tooth-blades of the upper 'lip' (*de* and *htp*, fig. 4, Pl. 7). These blades, as Noble (1927) has shown, fail to reach the posterior edge of the lip, so that a band of less cornified epidermis stretches for a short way behind them and forms the anterior margin of the mouth (*ul* and *mo*, fig. 5, Pl. 7).

The ? levator mandibulae posterior profundus muscle (*lmpm*, figs. 4, 5, Pl. 7; figs. 6, 7, Pl. 8; figs. 8, 9, 10, Pl. 9; figs. 11, 12, 13, 14, Pl. 10) is mainly inserted on the lower jaw. Its most medial fibres pass into a tendon which is inserted on the roof of a diverticulum of the mouth cavity underlying the lower jaw (fig. 4, Pl. 7, left side, unlabelled). Its most antero-dorsal fibres pass into a diffuse tendon (*lst*, fig. 3, Pl. 6) which concentrates in front into a weak strand inserted partly on the dorso-medial edge of the supra-rostral wing (*lst*, fig. 3, Pl. 6, right side) and partly on a septum of a lymph space of the snout.

The ? levator mandibulae posterior superficialis muscle (*lmsm*, fig. 3, Pl. 6; figs. 4, 5, Pl. 7; figs. 6, 7, Pl. 8; figs. 8, 9, 10, Pl. 9; figs. 11, 12, 13, Pl. 10) passes wholly into a similar diffuse tendon (*lt*, fig. 3, Pl. 6, right side) which makes rather vague connexions with the supra-rostral wing and the lymph space septum; the muscle has no well-organized tendon at all.

Neither of the tendons from these two muscles to the supra-rostral wing is strong or well marked and it is uncertain whether they could transmit an effective pull from their muscles to move the cartilage. Perhaps, when the lower jaw is tightly closed, the lateral flange on the posterior jaw cartilage may lever up the trabecular-quadrangle ligament and so pull the upper end of the supra-rostral wing backwards and upwards. The middle-piece of the larval upper jaw system is rigid and is unsupplied with muscular insertions.

Two ligaments are also attached to the larval upper jaw system (see fig. 19, Pl. 13, *tpl* and *lq*). The 'trabecular quadrangle ligament' (*tpl*, = *ligamentum cornu-quadratum laterale*,

of Schulze, 1892) runs from the antero-lateral tip of the quadrate to the postero-dorsal tip of the lateral supra-rostral wing. In *Rana temporaria* this same ligament passes further forwards to the antero-lateral tip of the trabecular horn and is independent of the supra-rostral (Pusey, 1938, fig. 8, Pl. 9). In *Discoglossus pictus*, as in *Ascaphus*, it is attached to the wing of the supra-rostral, but no longer to its postero-dorsal tip, but more anteriorly on its outer side nearer to the tip of the trabecular horn (personal observations). Thus *Discoglossus*, in this as in many other points, is intermediate between *Ascaphus* and *Rana*. In *Pelobates* it is attached, at the same time, both to the trabecular horn and to the adrostral prong of the supra-rostral (see Luther 1914, fig. 76).

The second is the ligamentum quadrato-ethmoidale (*lq*, fig. 19, Pl. 13). This runs forwards, embracing the lower edge of the pterygoid process; where this process ends in front, the ligament continues freely forwards below the internal nostril (fig. 4, Pl. 7), and is inserted on the upper part of the posterior pillar (*clsm*, fig. 18, Pl. 12), where the medial part of the supra-rostral is fused to the trabecular flange. In both *Rana* and *Discoglossus* this ligament is inserted on the under side of the trabecular horn, mid-way along its length, in the latter, to a special ventral flange which is thus probably homologous to the trabecular flange of *Ascaphus* (personal observation, and see also Litzelmann, 1923, figs. 12 and 13, for *Bombinator* and van Seters, 1922, fig. 3, Pl. 8, for *Alytes*).

(c) The Nasal-sac and Preoral Buccal Cavity.

The external nostril opens on a projecting funnel (Noble, 1927, fig. 8) at the level of the olfactory foramen. This funnel opens into a simple, undivided nasal-sac lying largely behind the olfactory foramen. The sac tapers behind into a membranous 'posterior narial tube' (*pnt*, fig. 3, Pl. 6; fig. 4, Pl. 7) which runs backwards and turns medially through the notch (*nnt*, fig. 18, Pl. 12; fig. 19, Pl. 13) to join the buccal cavity. This notch is closed below by the quadrato-ethmoidal ligament. The internal nostril (fig. 4, Pl. 7) opens by a valvular opening into a preoral buccal cavity which is housed between the cranial

floor and the median part of the supra-rostral system (*pm*, fig. 3, Pl. 6; fig. 4, Pl. 7; see also fig. 18, Pl. 12). Each wall of this preoral cavity is thickened into a longitudinal band of deep, ciliated epithelium (*eb*, fig. 3, Pl. 6; fig. 4, Pl. 7), supplied by a branch of the olfactory nerve (*lb*, fig. 3, Pl. 6) which runs backwards with the posterior narial tube and passes with it through the notch. *nnt*. Noble (1927, pp. 65 and 66) has described how the tadpoles of *Ascaphus* feed on particles taken in with a current of water through the nostrils and nasal-sacs and passed out at the median 'spiracle'. It is possible, therefore, that these ciliated bands may play a part in this method of feeding. Attempts to find further traces of a ciliary mechanism, however, have failed.

(d) The Side Wall of the Neurocranium and its Foramina.

In the absence of embryological evidence from earlier stages, the cranial wall only calls for a simple description; the various figures show the arrangement very clearly. The cartilage is thick and heavily built for so young a stage and the side wall is complete and without membranous tracts. The olfactory foramen, *at*, at its anterior end, has already been mentioned (p. 109). The optic and oculomotor foramina (*fon* and *focn*, fig. 19, Pl. 13) open into a common external pit in the side wall where the cartilage is thinner; a lightly built pila metoptica separates them. There is no separate foramen for the IVth nerve, since it passes out via the oculomotor foramen, which is a unique state of affairs, not found in the other frogs nor in the Urodeles. The pila antotica and parts of the oculomotor and trigeminal (*ft*) foramina are covered, in lateral view, by the broad ascending process of the palatoquadrate, which is fused to the orbital cartilage above the pila (see fig. 19, Pl. 13, where the limits of the trigeminal foramen and the anterior border of the auditory capsule are shown by broken lines, - - -). Fig. 8, Pl. 9, shows the relations in this region. The trigeminal foramen is an oval, vertically-lengthened opening between the auditory capsule and the side wall of the skull. Its anterior outlet is partially obstructed by two sheets of cartilage which lie across it from the

palatoquadrate to the cranial wall. These are the ascending process (*pa*) above and the commissura quadrato-cranialis anterior (*cqa*) below, both of which, of course, belong to the palatoquadrate. Thus there are three exits from the trigeminal foramen: (1) an upper outlet (*fid*, fig. 9, Pl. 9; fig. 15, Pl. 11; figs. 19, 20, Pl. 13; fig. 22, Pl. 14) between the capsule, the orbital cartilage, and the posterior edge of the ascending process; nerves V 2 and 3 and a lateral line branch of VII (fig. 9, Pl. 9; fig. 23, Pl. 14) pass through this opening; (2) an anterior outlet *pt*, fig. 17, Pl. 12) into a tunnel between the ascending process above, the pila antotica medially and the commissure and palatoquadrate ventrally and laterally (fig. 8, Pl. 9); the anterior end of this tunnel is seen in fig. 20, Pl. 13, marked *pt*. The tunnel transmits the profundus branch of the trigeminal nerve and a branch of the lateral head vein (fig. 8, Pl. 9); (3) a larger, ventrally-facing, pear-shaped opening, seen in fig. 17, Pl. 12, as a darkly shaded space on the left side, bounded behind by the front edge of the basitrabecular process (*bt*), medially by the trabecula, antero-laterally by the posterior edge of the commissure (*pcqa*), and laterally by the 'posterior basal process' (*bpr*). This last opening is the only representative, in the larval skull of *ASCAPHUS*, of the huge subocular vacuity of the larval skulls of all other frogs. It is important for the subsequent discussion (p. 154) to realize that no structures, such as nerves or blood vessels, pass upwards or downwards through this small vacuity and that, therefore, the posterior edge of the commissure (*pcqa*) could have been directly applied to the whole length of the basitrabecular process (*bt*), in the earlier ancestor, without affecting any other anatomical relationships. In such a case the vacuity would have been entirely absent.

From behind the notch (*nnt*, fig. 19, Pl. 13) for the internal nostril, to the posterior edge of the commissure (*pcqa*, fig. 15, Pl. 11; fig. 17, Pl. 12) the ventro-lateral edge of the trabecular cartilage is carried down into a flange which is fused (1) to the upper edge of the pterygoid process of the palatoquadrate

anteriorly (fig. 5, Pl. 7; fig. 6, Pl. 8) and (2) to the body of the quadrate itself more posteriorly (fig. 7, Pl. 8; fig. 8, Pl. 9). This fusion represents the commissure of modern-type frogs, but in a very much extended and probably more primitive condition.

(e) The Floor of the Neurocranium.

Even in so young a larva the floor of the cranium is fully chondrified into quite a thick sheet of cartilage; it is pierced by the two pairs of foramina typical of all frog larvae. Each foramen caroticum primarium (*fcp*, fig. 17, Pl. 12) admits the carotid artery of its side to the braincase, and each foramen cranio-palatinum (*fc*) transmits the palatine branch from it to the palate (figs. 2, 3, Pl. 6; figs. 4, 5, Pl. 7; figs. 6, 7, Pl. 8; fig. 8, Pl. 9). Behind the primary carotid foramina there is a slight pituitary fossa (*pitf*, fig. 20, Pl. 13), behind which again the cranial floor is thickened. In this thicker part the notochord (*n*, fig. 17, Pl. 12) is largely encased; however, its anterior tip is not surrounded by cartilage below, and this also holds true in the occipital region (fig. 17, Pl. 12). Elsewhere, the notochord is sheathed in cartilage both above and below, so that in this character *Ascaphus* is unlike both the Urodela and the other Anura (de Beer, 1937, p. 460); see figs. 12 to 14, Pl. 10. The cranial floor in the auditory region will be described in the following section.

(f) The Auditory Capsule.

The relations of the anterior end of the capsule are well shown in section in figs. 9, 10, Pl. 9; figs. 11, 12, 13, Pl. 10; and are reconstructed in figs. 22 and 23, Pl. 14, among others.

The auditory region of the adult skull of *Ascaphus* has been described by de Villiers (1934). A redescription from his account was given by Pusey (1938) with a reconstruction made from his original figures. However, some doubt remained about the truth of my interpretation given there; this doubt is now removed by the present research, which shows that interpretation to be correct.

It is undoubtedly correct that there is a complete basitra-

becular process (*bt*), projecting laterally from the cranial base, in front of the foramen (*pf*) for the palatal branch of the facial nerve. This process projects beyond the floor of the auditory capsule as a free 'cartilage ledge of the auditory capsule' (de Villiers; see fig. 10, Pl. 9; fig. 11, Pl. 10; and fig. 22, Pl. 14, of this paper). To its outer end is fused the 'posterior basal process' (*bpr*); see discussion on pp. 154 et. seq. Part of the projecting plate lies behind the exit of the palatine nerve and is thus a post-palatine commissure (*ppc*, fig. 12, Pl. 10). Both parts of the plate form the effective floor of the anterior part of the auditory capsule. The true floor of the capsule may be seen above it, however, as a membranous sheet (*mfac*, figs. 11 and 12, Pl. 10) covering the branches and ganglion of the facial nerve and parting them from the auditory nerve above. More anteriorly, the cartilagenous wall and floor of the capsule overhang and are fused with the basitrabecular process in front of the hyomandibular branch of the facial nerve. This nerve, therefore, runs out through its own lateral foramen, above the process (fig. 23, Pl. 14; figs. 11, 12, Pl. 10). In the same way the antero-medial section of the vertical capsular wall is also absent and the deficiency is made good by the absorption into the capsule of the prefacial commissure (*pfc*, fig. 10, Pl. 9; fig. 11, Pl. 10; fig. 15, Pl. 11; fig. 22, Pl. 14) which lies in front of the joint exit of nerves VII and VIII. How far forwards the commissure may extend and at what point it is fused to the true capsular wall cannot be determined at this late stage of development. Its presence, of course, is proved by the fact that it separates the root of the Vth from the root of the VIIth nerve and makes the VIIth nerve appear to cross the apparent capsular cavity lateral to it. These characters show a marked similarity to the condition in Urodeles, as was pointed out by de Villiers (see Goodrich, 1930, pp. 259 and 278, and de Beer, 1937).

Only a short statement will be made about the remainder of the auditory capsule, as this has already been described in the adult condition by de Villiers. Its ventro-lateral wall is pierced by the fenestra ovalis (*fo*), which is closed by a plate of dense mesenchyme (figs. 13, 14, Pl. 10; fig. 17, Pl. 12; fig. 19, Pl. 13).

No operculum has yet been formed and there is no trace of any columella system; this latter, of course, is also absent from the adult skull (de Villiers), although the operculum is present. The medial wall of the capsule is very well formed for a young larva. It is well shown in fig. 15, Pl. 11. From the front backwards, it is pierced by: (1) an anterior acoustic foramen (*foa*) (or more exactly a joint opening to this foramen and to the tunnel for the facial nerve); (2) two small median acoustic foramina (*fam*); de Villiers finds only one in the adult skull; (3) an endolymphatic foramen, more dorsally (*fen*); (4) a superior perilymphatic foramen (*fps*); and (5) an inferior perilymphatic foramen (*fpi*). Just antero-laterally to this last foramen, the true floor of the auditory capsule is pierced by a large opening, the fenestra rotunda. However, there is no through way out of the apparent capsular cavity to the subcranial space below, because the cranial basal plate is carried out as a ventral floor whose outer edge is fused to the lateral wall of the capsule. Thus the fenestra rotunda opens into a small extra-capsular space, from which, however, there is no ventral outlet. This cavity has the false appearance of being a part of the true capsular cavity, and the inferior perilymphatic foramen opens laterally into it. There is no preoccipital arch in front of the jugular foramen, whilst the occipital arch is well developed and is fused to the side wall of the auditory capsule in the usual way. The relations of the auditory capsule to the palatoquadrate will be dealt with later on (pp. 119 to 121).

(g) The Cartilage Cranial Roof.

There is no complete tectum synoticum as yet, although the anterior border of it is probably represented at the sides by the two cartilage projections, *ts*, figs. 12, Pl. 10, and fig. 15, Pl. 11; a complete arch is present in the adult (de Villiers). There is no taenia tecti medialis nor *t. t. transversalis*; these are also absent in the adult. The sides of the roof are formed by the slightly overhanging upper edges of the orbital cartilages, which pass back into the heavily built flanges of the taeniae tecti marginales (*tm*, fig. 10, Pl. 9; fig. 11, 12, Pl. 10), which project inwards from the upper edges of the capsules.

(h) The Palatoquadrate.

In all modern-type Anuran larvae, the articular region of the quadrate lies far forwards in the snout, below the olfactory foramen, but in *Ascaphus* it is no further forward than the region of the pila metoptica, between the optic and oculomotor foramina. All the parts of the quadrate bar lie correspondingly far back and all are very heavily built, whilst the attachments to the cranium show a most exaggerated autostylism. The absence of any obvious subocular vacuity between the cranial wall and the palatoquadrate also differentiates *Ascaphus* from all other frogs.

(i) The Commissura Quadrato-cranialis Anterior.

This quadrato-cranial connexion (*cqa*) is present as a greatly extended flange fused to the ventro-lateral edge of the trabecula, from the level of the notch (*nnt*, fig. 19, Pl. 13) for the internal nostril to a position close in front of the anterior capsular face; this posterior limit is shown as a darkly shaded line in fig. 17, Pl. 12, and as a dotted line (.....) in fig. 15, Pl. 11, and is marked in each case as *pcqa*. Fig. 5, Pl. 7; figs. 6, 7, Pl. 8; fig. 8, Pl. 9; and fig. 17, Pl. 12, show clearly that the anterior half of the commissure acts as a support between the trabecula (*t*) and the dorsal surface of the pterygoid process of the palatoquadrate (*ptc*). Such a clearly marked pterygoid process is a unique feature in *Ascaphus*; in all other frog larvae the process is obscured in the body of the quadrate owing to the more anterior position of the pars articularis (Pusey, 1938, p. 545 and Text-fig. 7E of this paper.) The posterior half of the commissure joins the trabecula to the body of the quadrate, behind the pars articularis. About half of this posterior section of the commissure is covered from above by the ascending process (*pa*, fig. 15, Pl. 11) and it is this fact which, in part, accounts for the apparent absence of any subocular vacuity when the skull and jaws are viewed from above.

(ii) The Posterior Spur of the Quadrate.

A solid spur of cartilage projects ventrally from the under side

of the quadrate bar, behind and somewhat medially to the pars articularis. The lower jaw abuts against it from in front, and the tip of that part (*saq*, fig. 21, Pl. 14) of the ceratohyal (*ch*), which articulates with the quadrate, abuts against it from behind (fig. 19, Pl. 13). This spur is presumably the homologue of a similar spur described in *Rana* (Pusey, 1938, p. 504).

(iii) The Pterygoid Bone-rudiment (= ligamentum quadrato-ethmoidale).

Starting posteriorly, close to the inner side of the spur (fig. 7, Pl. 8) and continuing forwards along the under side of the pterygoid process (fig. 5, Pl. 7; fig. 6, Pl. 8) there lies a band of dense ligamentous tissue (*lq*) which is almost certainly the rudiment of the pterygoid bone, although this will require final confirmation in older stages; it is also certainly the equivalent of Gaupp's quadrato-ethmoidal ligament of *Rana*. Where the pterygoid process ends in front, the ligament becomes free and forms the ventro-lateral border of the notch (*mnt*) for the internal nostril. It passes forwards to its insertion on the conjoined trabecular horn and supra-rostral system. Fig. 19, Pl. 13, shows the full length of this ligament (*lq*), part of which is dotted, where it passes behind the lower jaw.

(iv) The 'Posterior Basal Process' of the Quadrate.

Behind the spur, the body of the quadrate is drawn down into a ventral keel, whose under surface is grooved with a longitudinal notch (*nc*, fig. 17, Pl. 12), into which the upper edge (*saq*, fig. 21, Pl. 14) of the ceratohyal is fitted; in other frogs this same groove lies transversely. As this keel passes backwards, it becomes more and more detached from the body of the quadrate and finally projects posteriorly as a free process, the 'posterior basal process' (*bpr*, fig. 10, Pl. 9; fig. 11, Pl. 10; fig. 17, Pl. 12), which is fused behind to the ventral side of the antero-lateral, free end of the basitrabecular process (*bt*, figs. 11, 12, Pl. 10; fig. 17, Pl. 12; fig. 19, Pl. 13). It will be seen from fig. 17, Pl. 12, that the posterior edge of the commissure (*pcqa*) passes laterally and becomes the inner border of this posterior basal process and therefore delimits and encloses the small

subocular vacuity in front. An articulation between a posterior basal process of the palatoquadrate and the outer end of a complete basitrabecular process from the cranial floor is, in *Ascaphus*, a feature unique among frogs. In *Discoglossus* such a basal process is still present in miniature (Pusey, 1938, p. 530, footnote, and Text-fig. 6, *bpr*, of this paper), but only the outer end of the basitrabecular process is present in the form of a pseudobasal process, from metamorphosis onwards. All other frogs, including the other members of the family *Discoglossidae*, have lost this posterior basal process.

(5) The Ascending Process and Larval Otic Process.

From the dorsal side of the body of the quadrate the ascending process (*pa*) rises up as a broad, flat strap of cartilage which slopes inwards and upwards to become fused to the orbital cartilage at the top of the pila antotica (*pia*, fig. 8, Pl. 9; fig. 15, Pl. 11; fig. 19, Pl. 13); it forms the roof of a 'profundus tunnel', as explained on p. 114. Nerves V 2 and 3 pass out behind and above it, from the dorsal division of the trigeminal foramen (fig. 9, Pl. 9; fig. 23, Pl. 14) whilst V 1 passes forwards below it, in the tunnel. The posterior borders of both the ascending process and the general body of the quadrate are fused to the anterior tip (*tac*) and to the side-wall (*f*) of the auditory capsule (see particularly figs. 9, 10, Pl. 9; figs. 11, 12, Pl. 10; fig. 22, Pl. 14); *oac*, in fig. 15, Pl. 11, and *f*, in fig. 19, Pl. 13, show the line along which the quadrate is fused to the capsule. This fusion is not the larval otic process but is a unique feature in *Ascaphus*, helping to give an increased autostylic support to the quadrate bar. A similar state of affairs, however, is probably present in the larval skull of *Xenopus*, though this has not previously been recognized (see p. 171). In *Discoglossus*, the back of the quadrate fits on to the tip and the side of the capsule by a loose, ball and socket joint; yet there is no fusion of cartilage between them. Farther back at the side of the auditory capsule, in *Ascaphus*, this fusion is

interrupted at one point by a foramen (*fvcl*, fig. 22, Pl. 14, and in the other reconstructions) which allows a branch (*vclb*) of the lateral head vein to pass downwards from the overlying jaw muscles to join the main venous trunk (*vcl*) below (see fig. 13, Pl. 10). That part of the quadrate which lies behind this foramen and which is again fused to the capsular wall, is the larval otic process (*pot*, all reconstructions). See discussion, p. 167.

(vi) The Muscular Process.

The muscular process of the quadrate, so typical of all other frogs, is scarcely present in *Ascaphus* as a well-developed structure. It is, in fact, represented by the whole extent of the lateral, upper border of the body of the quadrate from the quadrate tunnel (*at*, left side of fig. 6, Pl. 8) to the larval otic process, inasmuch as it is this part which gives origin to hyoid muscles (*ohm*) to the ceratohyal, and (*sam*) to the jaw (fig. 5, Pl. 7; figs. 6, 7, Pl. 8; figs. 8, 9, 10, Pl. 9; figs. 11, 12, 13, 14, Pl. 10). These facts fully bear out the argument previously given (Pusey, 1938, p. 523, &c.) that the whole outer border of the larval quadrate bar, from the larval otic process behind, to the front border of the muscular process in front, is but an elongation of the shorter process seen in less modified Vertebrate types. The more obviously upraised anterior part of the border of the quadrate of *Ascaphus* is marked as *pmq*, in figs. 19 and 20, Pl. 13, and in the sections shown in fig. 7, Pl. 8, and fig. 10, Pl. 9. See discussion, p. 168.

(vii) An Arterial Tunnel through the Quadrate.

Finally, attention must be drawn to a tunnel (*at*), which passes through the body of the quadrate from behind forwards, below the muscular process. This is well shown in the reconstructions, particularly in figs. 19 and 20, Pl. 13, and in the sections drawn in figs. 6 and 7, Pl. 8. It transmits a branch (*cbr*) of the carotid artery, which supplies the anterior ends of the adductor jaw muscles. It is a unique feature in *Ascaphus*. See discussion, p. 173.

(i) The Lower Jaw System.

The lower jaw is seen in lateral view in fig. 19, Pl. 13, in

dorso-lateral view in fig. 21, Pl. 14, in anterior view in fig. 20, Pl. 13. and in section in figs. 4, 5, Pl. 7, and figs. 6, 7, Pl. 8. As in most other frogs, the larval jaw is divided into four cartilages. The anterior jaw cartilages (*ajc*) call for no special description; they are small rods held together medially by a copula of dense mesenchyme and their outer ends articulate with the inner borders of the posterior cartilages. In front of them the epidermis is cornified into a single, minute tooth-blade. The posterior cartilages, on the other hand, are large, heavily built plates. The notable features about them are their great antero-posterior extension and the postero-ventral spurs which they carry on their under sides. These two features assist in increasing the degree to which the floor of the pharynx is walled in by cartilage and are probably adaptations helping to resist the pressures set up when the sucker system is in use.

The huge ventral adductor jaw muscles (*lmpm*) have most extensive insertions on the whole concave upper faces of these cartilages; assisted by the ventral spurs, they probably play an important part in the peculiar method of progression used by the larva, whilst it remains adhering to the substratum by its sucker. The development of the excessive autostylism of the whole upper jaw system is probably an evolutionary response to meet the strains set up by these heavy muscles.

(j) The Hyo-branchial Apparatus.

In the head of all modern-type frogs so far described many structures, of which the hyo-branchial apparatus is one, have moved forwards into the snout, see pp. 146. Consequently in these frogs the posterior ends of the gill bars, which lie well in front of the occipital region of the skull, are wholly hidden by the skull in dorsal view. In the Urodeles, however, the ends of the bars are clearly visible for some distance behind the occiput. This is true also of *Ascaphus*, as will be seen if fig. 15, Pl. 11 is superimposed on fig. 21, Pl. 14, so that the lower jaw in each is made to coincide. It is thus true to say that the whole hyo-branchial apparatus lies much farther back in *Ascaphus* than in any other frog.

A comparison of fig. 21, Pl. 14, of this paper with figures of

Gaupp's reconstruction of the larval hyo-branchial apparatus of *Rana temporaria* (Gaupp, 1904, and as copied in many text-books, e.g. Goodrich, 1930, fig. 471) brings out a number of differences and points of interest. Of great importance is the distortion which must have taken place progressively in the evolution of the Ranid and modern-type condition, from the more primitive condition which *Ascaphus* has retained and shares with the Urodeles. This distortion extends to the ceratohyal and to each of the branchial bars behind it. The evolutionary change has entailed the movement forwards, in successive ontogenies, of the centre of each cartilaginous arch relative both to its lateral (dorsal) end and to its medial (ventral) attachment to the hypobranchial plate (*hbp*). Thus, in *Ascaphus*, each arch is somewhat concave along its anterior edge, whilst in *Rana*, each is convex anteriorly. This change of shape becomes more marked on passing from the last branchial arch behind to the ceratohyal in front. In fact, the change is so great in the ceratohyal, that whereas, in *Ascaphus*, this bar projects postero-laterally at an angle of about 45° to the long axis of the head, in *Rana* it has become a short bar, lying transversely across the pharynx floor, with its axis almost at right angles to the axis of the head. In *Ascaphus* the upper edge of the bar (*saq*) articulates with a longitudinal groove in the under side of the quadrate (*nc*, fig. 17, Pl. 12), whilst in *Rana*, this groove has become transversely placed (Pusey, 1938, *nc*, fig. 1, Pl. 33). This rotation in the modern-type frog is correlated with a shortening in length, which prevents the outer end of the ceratohyal from projecting far out beyond the overlying quadrate bar in a way that would upset the stream-lining of the whole larval head. It is shown on p. 168 that this rotation and shortening is also correlated with the development of a tall muscular process on the quadrates of modern-type frogs; thus the presence of a muscular process is but a further aspect of the general forward migration of the splanchnic structures.

Fig. 21, Pl. 14. should be self-explanatory. It is only necessary to stress those characters in which *Ascaphus* differs from *Rana*. In *Ascaphus* the whole hyo-branchial apparatus

is more robustly built and more strongly consolidated. The inner borders of the ceratohyals overlap the basibranchial copula (*bbc*, figs. 9, 10, Pl. 9; fig. 11, Pl. 10), whilst their posterior processes are fused to the bases of the 1st branchial arches (*fu*, fig. 21, Pl. 14). The whole basibranchial copula (*bbc*) is much more heavily built, and the two hypobranchial plates are fused to it and are not separated from one another in the middle line behind. All these characters make for solidity.

The ventral end of the first ceratobranchial joins the tip of the posterior process of the ceratohyal, and together these two run back to join the base of the second ceratobranchial; all three are then fused to the edge of the hypobranchial plate. The third ceratobranchial is separately fused to this plate farther back, whilst the fourth arch is entirely independent of it, its ventral (anterior) tip ending freely in a ventral position (see right side). This freedom of the fourth bar is not found in other frogs, but is a character shared with the Urodeles.

Dorsal spicula (*sp II* and *III*) are present on the edges of the hypobranchial plate, overlying the points of fusion of the second and third ceratobranchials; the first pair of the series, as found in *Rana*, is absent. What appears to be the fourth of the series is a pair of long, strong, rounded cartilages, firmly fused to the hypobranchial plate and reaching back to the tiny glottis. Personal observations on victoria-blue preparations and sections of the hypobranchial apparatus of *Rana temporaria* show that the fourth spicula make up a considerable part of the thyroid processes of the adult, being combined with what remains of the hypobranchial plates and being added to by some new cartilage growth. It is likely, therefore, that the much larger fourth spicula of *Ascaphus* will also be found to make up the thyroid processes of the adult. The adult 'hyoid' apparatus has been figured by Frazier (1924, fig. 11, Pl. 2). It possesses well developed thyroid processes, each, however, with a forked end. *Liopelma hochstetteri* has equally well developed, but unforked, processes (Trewavas, 1933, fig. 4). Thus the terms 'thyroid process' and 'fourth spiculum' are probably largely synonymous.

But this is not all. In *Ascaphus* each fourth spiculum is supplied with a subarcualis rectus muscle (just as the IVth arch is) and with a subarcualis obliquus muscle (like the IVth, IIIrd, and IInd arches). Therefore, the possibility should be kept in mind that the 4th spiculum is really a Vth branchial arch, still quite well developed in *Ascaphus* and supplied with muscles like the other arches. In *Discoglossus* and *Rana* there is no larval muscle supply to the fourth spiculum and the whole structure is relatively insignificant.

I hope to make further studies and to publish figures of the relations of this probable Vth arch to the surrounding gill clefts, muscles, and nerves. Should this suggestion prove to be correct, *Ascaphus* would become further noteworthy as being the only living tetrapod to retain a recognizable Vth branchial arch.

Each arch has a solid, rounded ventral part, which expands on passing backwards, into a broad, flat plate, which is quite heavily built and has (at least as yet) a smooth outline, without the ragged branchial rays typical of *Rana* and other frogs. The under sides of the IInd and IIIrd arches are not joined by any processus branchialis, such as is found in *Rana*. This junction is also absent in fully formed larvae of *Discoglossus*, although here a long process reaches forwards from the IIIrd arch towards the IInd, with which, however, it is still unfused. In this small point, too, then, *Discoglossus* is intermediate between *Ascaphus* and *Rana*.

Each gill bar passes dorsally and Nos. I to III have bifurcated and inrolled, dorsal ends which overlap one another. The posterior prong of each anterior arch overlaps the anterior prong of the arch behind; in these places the arches are fused to give terminal commissures (*tc*, I-II, Text-fig. 4, p. 140), though this is not apparent from fig. 21, Pl. 14, except in the case of the IIIrd and IVth arches. The dorsal end of the Ist arch carries a long, forwardly directed dorsal process (*dpha* I) underlying the otic region of the palatoquadrate (figs. 13, 14, Pl. 10, and Text-figs. 1 and 2, pp. 132 and 133). (Note: the IIIrd bar, on the right side, is presumably atypically developed

and is so deeply bifurcated that its dorsal end is split into two independent parts.)

The under side of the basibranchial copula (*bbc*) is carried downwards and backwards into a wide ventral keel, which divides behind into two slightly diverging prongs (*upbc*), which become free of the plate and underlie the hypobranchial region. These prongs are seen in section in fig. 12, Pl. 10, and in Text-fig. 1, p. 132. They are the homologue of the 'urobranchiale', a process of the basibranchial copula (copula II) of the Urodeles and have many of the same relations to structures such as muscles, thyroid glands, and ventral aorta. Thus, their tips embrace the conus region of the heart and the vertically placed ventral aorta (*va*), whilst their outer borders give attachment to the subarcuales obliqui (*saom II* to *I'*) and the recti cervicis muscles (*rcm*); the thyroid glands (*thy*) lie above them. Such a well-marked urobranchial element is unique among frogs; usually there is a small, undivided knob on the under side of the copula.

5. A COMPARISON OF CERTAIN CRANIAL MUSCLES OF ASCAPHUS, URODELA, AND OTHER ANURA.

(a) Preface.

It was not my original intention to give here an account of the cranial muscles of the larval *Ascaphus*. A study of the jaw and branchial cartilages, however, showed many unique features in the related musculature and revealed that, in certain respects, *Ascaphus* shows a considerable similarity to the Urodeles, whilst, in other respects, it is more primitive either than that group or the Gymnophiona. I hope, at a later date, to be able to publish figures of the cranial muscles of *Ascaphus*, compared with the muscles of Urodeles, *Discoglossus* and *Rana*, but, in view of the war situation, I have decided to set down the facts already worked out and give here a simple account of the muscles of *Ascaphus* compared with those of certain other Amphibians.

I have not had time to study, in detail, all of the rather extensive and involved literature on Amphibian musculature. I have relied, therefore, largely on the account given in Edge-

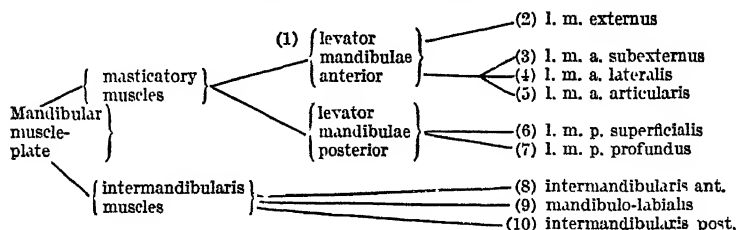
worth's (1935) monograph on 'The Cranial Muscles in Vertebrates', particularly as his tables of synonyms give great help with the earlier literature, to much of which, however, I have referred for special points. For convenience I have used Edgeworth's nomenclature, so far as is possible, but this does not mean that his interpretations have necessarily been accepted. It is clear from what follows that certain of his generalizations must be considerably modified, in view of the findings from *Ascaphus*. In addition to Edgeworth's book I have found a more recent paper by Eaton (1936), on the Urodele *Discamptodon ensatus*, to give a clear statement of the cranial muscles of the Amblystomid Salamanders compared with the muscles of other Urodeles.

(Chiefly, however, the following accounts are based on personal observations on various developmental stages of *Amblystoma tigrinum*, *Salamandra maculosa*, *Ascaphus truei*, *Discoglossus pictus*, *Bombina variegata*, and *Rana temporaria*. No detailed discussion of the muscles will be given at the present time, but points of particular significance will be stressed as they arise.

(b) The Mandibular Muscles.

Modern-type Frogs in General.

Edgeworth (1935), following earlier work, identifies ten pairs of mandibular muscles in the typical, modern-type frog larva. They are given in the following diagram, which aims at showing their origin in development (after Edgeworth's account).



(This list takes no account of the levator bulbi muscle which is a further outgrowth from the l. m. anterior muscle.)

Ascaphus.

In *Ascaphus* only five pairs of muscles are independently represented; these are probably nos. 5 (or ? 2), 6, 7 (or 7 ? + 1), 8, and 10. This absence of subdivision of the muscle-blocks into small muscles which, in the modern-type frogs, are inserted on the supra-rostral cartilage (nos. 2, 3, and 4) and on the larval lips (no. 9) would seem to be a primitive character in *Ascaphus*. There is, however, some difficulty in establishing homologies when the early development is not known, whilst names, which are devised for muscles in a subdivided condition, often cannot be accurately applied to the muscles of a simpler system. Consequently some of these names have been used guardedly for the muscles of *Ascaphus* and these are preceded by a question mark. The choice of names has been guided by the general topography of the muscles and by the nature of their origins and insertions. As an example of the type of difficulty, we may take the case of no. 5, the ? l. m. p. articularis muscle. This muscle, in *Ascaphus*, has just the position, origin, and insertion of the muscle of that name in the advanced frogs, and so this name has been used for it; but it may well be that it is actually also the rudiment of muscles nos. 3 and 4 as well as 5. There is also a further alternative. It seems to me not impossible that this muscle may prove to be no. 2, the l. m. externus muscle, which, in development, has grown back to the otic process (= anterior edge of the muscular process) below nerve V 3, instead of above it; this could have possibly come about through nerve V 3 being carried far up dorsally on the huge mass of the adductor muscles of the jaw. The advantage of this theory, which can only be proved by a study of the early development, is that it brings *Ascaphus* into line with the Urodeles, all of which possess a large l. m. externus muscle but none of the muscles 3 to 5, which are typical of the frogs. But again it must be stressed that the relations of the muscle in question to nerve V 3 are not those of the l. m. externus of the Urodeles. The relations in *Ascaphus* are clearly shown in fig. 5, Pl. 7, and fig. 6, Pl. 8 (*lman* and V 3). Also against the theory is the fact that the l. m. externus muscle

is absent in *Bombina* and is very poorly developed in *Discoglossus* of the *Discoglossidae*.

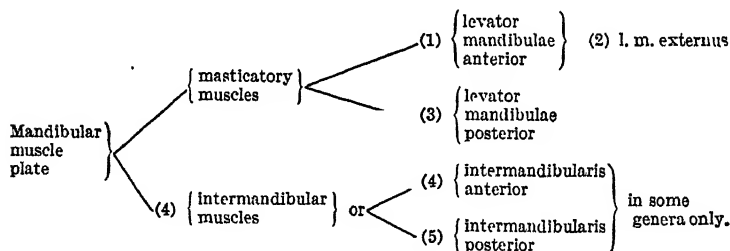
All the other muscles, except no. 8, the intermandibularis anterior, are well shown in fig. 3, Pl. 6; figs. 4, 5, Pl. 7; figs. 6, 7, Pl. 8; figs. 8, 9, 10, Pl. 9; figs. 11, 12, 13, 14, Pl. 10, and call for little description. The very great development of ?l. m. p. profundus should be noted; this is probably in part due to its use by the tadpole in connexion with the special sucker mechanism and mode of larval progression. Some part, however, of its great bulk is presumably due to the fact that there is no independent l. m. anterior muscle, whose substance is therefore probably represented in this muscle, thus increasing its size. The absence of an independent l. m. anterior is surprising, for such a muscle is present in all other *Amphibia*. It may be noted, however, that the most medial of the fibres making up the huge muscle block take origin from the orbital cartilage above and in front of the ascending process and therefore in front of the joint exit of nerves V 2 and 3; this can be seen in fig. 7, Pl. 8, and fig. 8, Pl. 9. The remaining fibres of the block arise from the quadrate and the roof of the auditory capsule postero-laterally to the nerves. These medial fibres have just the relations, then, of the l. m. anterior muscle of the *Urodeles*, and though not independent, presumably represent this muscle fused to the l. m. p. profundus muscle. The fibres concerned remain medial to the rest of the muscle block and are inserted on the roof of the mouth cavity and the median side of the jaw. They do not pass under the l. m. p. profundus muscle to attain a lateral position anteriorly, as is customary in all modern-type frogs except the *Pipidae*. In this respect again *Ascaphus* is like the *Urodeles*. *Ascaphus* is also unique among frogs in having such l. m. anterior fibres arising in the primitive position high up on the orbital cartilage in front of the two branches of the trigeminal nerve. In other frogs, where a large subocular vacuity has been evolved, the head of the muscle has moved downwards through the vacuity and arises on the front face of the auditory capsule below the ascending process and on its under side.

Another point of interest is found in the origins of the l. m. p. superficialis and profundus muscles, which lie far back over the auditory capsule. In all modern-type frogs these muscles arise wholly from the quadrate and therefore in a more anterior position. This posterior origin, in *Ascaphus*, is presumably related to the relatively posterior position of the lower jaw system, but whether it is a primitive character or not is hard to decide; see also p. 170.

The hinder edge of the intermandibularis posterior muscle underlies the front edge of the interhyoideus muscle (see fig. 9, Pl. 9). In this small point *Ascaphus* is like the Urodeles and unlike the other frogs.

Urodeles.

The mandibular muscles of Urodeles are present as follows (Edgeworth):



Rana temporaria.

Rana possesses all the muscles listed on p. 127; the l. m. a. lateralis, however, only develops just before metamorphosis and is essentially an adult muscle.

Discoglossus pictus.

All ten pairs of muscles are present in *Discoglossus*. L. m. externus is small and is absent on one side in one specimen. L. m. a. lateralis lies laterally, and not medially, to nerve V 3 and l. m. a. subexternus is divided into two slips. Thus, *Discoglossus* is in no way primitive in its mandibular muscles.

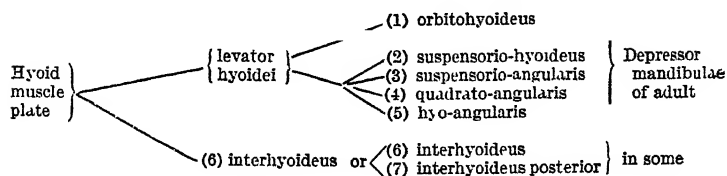
Bombina variegata.

In *Bombina* l. m. externus is absent; l. m. a. subexternus is in two slips, and l. m. a. articularis lies medially to V 3.

(c) The Hyoid Muscles.

Modern-type Frogs in General.

Edgeworth's description of the origin and form of the hyoid muscles of the modern-type Anura may be expressed as follows:



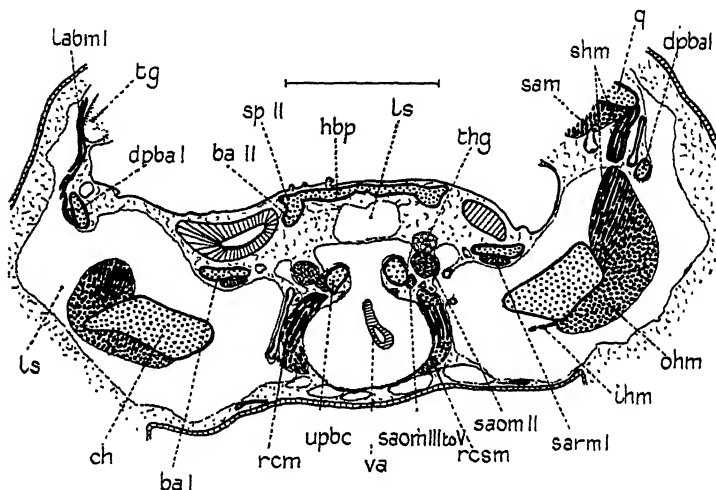
Ascaphus.

Of the above muscles *Ascaphus* possesses only four pairs, which, from their relationships, may be identified as nos. 1 (?+2), 3, 4, and 6: they are all clearly seen in fig. 5, Pl. 7; figs. 6, 7, Pl. 8; figs. 8, 9, 10, Pl. 9; figs. 11, 12, 13, 14, Pl. 10; and Text-figs. 1, 2, and 4 (pp. 132, 133, and 140), and are labelled as: (1) *ohm*, (3) *sam*, (4) *qdm*, and (6) *ihm*. In addition *Ascaphus* possesses a pair of muscles homologous with no. 3 of the Urodeles, i.e. the branchio-hyoideus externus muscles (*bhem*); see pp. 132 and 134 below.

There is no independent suspensorio-hyoideus muscle, though some of the postero-dorsal fibres (*shm*) in the block marked as the orbitohyoideus run a more vertical course from the larval otic process to the tip of the ceratohyal; they do not constitute a separate muscle, however. The hyo-angularis muscle is entirely absent, but the suspensorio-angularis muscle appears to have two heads, arising from the quadrate, one above and one below the hyomandibular branch of the VIIth nerve and a branch of the carotid artery (*VIIhm* and *cbr*, fig. 7, Pl. 8; figs. 8, 9, Pl. 9), yet the fibres form one muscle block only; if, however, the lower head shifted to the ceratohyal (fig. 9, Pl. 9) and the muscle divided into two, an independent hyo-angularis muscle would result.

A large orbitohyoideus muscle (*ohm*) is present, arising all along the upper, outer edge of the quadrate

bar, from its anterior tip beside the quadrate tunnel (at), almost to its otic region (figs. 6, 7, Pl. 8; figs. 8, 9, 10, Pl. 9; figs. 11, 12, 13, Pl. 10); it passes downwards and backwards, to be inserted on the end and outer side of the ceratohyal (Text-figs. 1 and 2). Similarly, the suspensorio-angularis muscle (*sam*) takes origin from the under side of the quadrate, right back to its larval otic



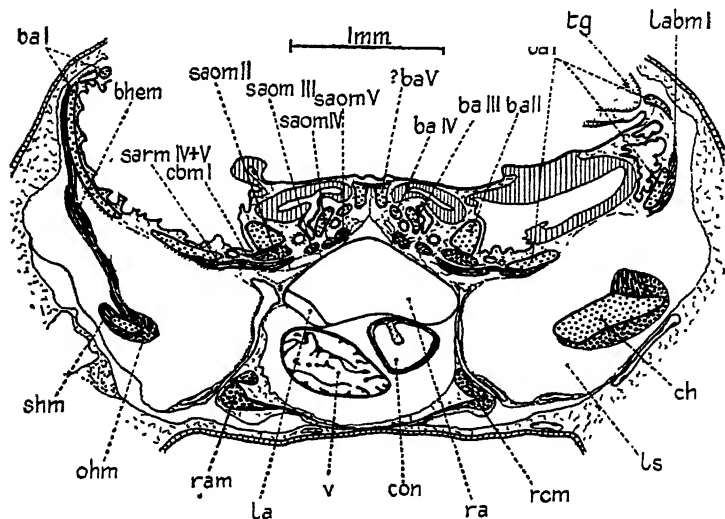
TEXT-FIG. 1.

Section 11-1-7, through line vv in fig. 21, Pl. 14. For key to lettering see p. 181.

process. Thus, neither this muscle nor the orbitohyoideus is restricted to a localized quadrate muscular process, as in the more advanced frogs. See discussion, p. 168.

Of some importance is the presence of a pair of branchiohyoideus externus muscles. Each is a moderately large bundle of fibres inserted on the posterior tip of the ceratohyal (*bhem*, Text-fig. 2), largely on its median side, behind and above the interhyoideus muscle and passing upwards and backwards, to arise on the outer side of the first ceratobranchial, near its postero-dorsal border and behind the insertion of levator I

(Text-fig. 4). These are the relations of this same muscle in the Urodeles, with the difference, that in *Ascaphus* the insertion of the muscle lies very far back on the tip of the ceratohyal and not along its whole under side as in the Urodeles. This muscle has not been described before in the Anura, though both *Discoglossus* and *Bombina*



TEXT-FIG. 2.

Section 11-4-6, through line ww in fig. 21, Pl. 14. For key to lettering see p. 181.

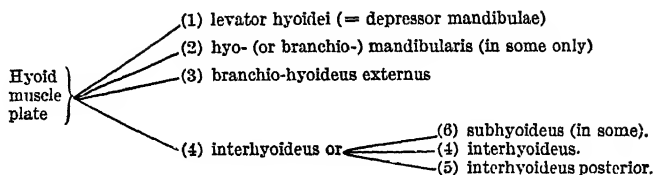
retain it as a small bundle throughout the larval period; in this respect again the *Discoglossid* frogs are intermediate between *Ascaphus* and the more advanced members of the group.

The interhyoid muscle does not extend back as far as the posterior tips of the ceratohyals, and there is no separate i. posterior muscle in the opercular wall.

The relative simplicity of the hyoid muscles on the one hand and the presence of the branchio-hyoid muscles on the other are obviously primitive characters in *Ascaphus*.

Urodeles.

The hyoid muscles, in form and origin, are as follows (Edgeworth):



Muscle no. 2 above seems to be wholly unrepresented in *Ascaphus* and the other frogs.

Discoglossus pictus.

Muscles 1, 2, 3, 4, and 6 of the anuran series are present in *Discoglossus*. No. 5, the hyo-angularis muscle, is absent as in *Ascaphus*, though it is present in *Bombina*. The interhyoideus posterior muscle is absent as in *Ascaphus* and *Rana*. The suspensorio-angularis and the suspensorio-hyoideus muscles take origin from the auditory region of the quadrate as in *Ascaphus* (where the orbitohyoideus \simeq the suspensorio hyoideus; see p. 131, above); this is unlike the conditions in the modern-type frogs, where the origins lie in the region of the muscular process. The quadrato-angularis muscle arises medially to the ceratohyal-quadrate articulation as in *Ascaphus* and not laterally to it as in *Rana*.

A small branchio-hyoideus externus muscle is present on each side as in *Ascaphus* and the Urodeles; it is also present in *Bombina*. Of this muscle Edgeworth (1935, p. 106) says: 'The Branchio-mandibularis externus' (*sic*, which from his p. 103 is clearly a misprint for 'branchio-hyoideus externus') 'of larvae of Urodela has no homologue in Dipnoi, Apoda, and Anura' and 'appears to be a secondary larval structure'. This statement clearly now needs revision.

From the above it is clear that *Discoglossus* retains many small primitive characters which it shares with *Ascaphus* and sometimes with the Urodeles.

Rana temporaria.

Rana possesses all the muscles nos. 1 to 6 of the anuran series, but no. 7, which is present in other genera. e.g. *Pelobates* and *Bufo*, is absent.

(d) The Levatores Arcuum Branchialium
Muscles.

Ascaphus.

Levator I (*labm I*, figs. 13, 14, Pl. 10; and Text-figs. 1 and 2) takes origin from the outside of the membraneous skull roof, over the otic process of the quadrate and runs downwards and a little backwards, to be inserted on the outer side of the first arch and on the base of its dorsal process (*dpha I*). It extends about a quarter of the way down the arch, in front of the efferent artery and the attachments of the branchio-hyoideus externus muscle and the first constrictor muscle.

Levator II arises from the cartilage wall of the lateral semicircular canal and from the sheath of the neck muscles and runs backwards and downwards to be inserted on the outer side of arch II, a quarter of the way down its posterior face in front of the efferent blood-vessel (*labm II*, Text-fig. 4).

Levator III arises postero-ventro-medially to L. II, from the wall of the posterior ampulla of the capsule and from the sheath of the neck muscles. It passes backwards horizontally and is then folded forwards as a <, round the back of arch III, and passes a little way along its under side.

Levator (+constrictor) IV takes origin wholly from the sheath of the neck muscles, under the back of the auditory capsule. It passes backwards horizontally to loop forwards again behind the last gill pouch and be inserted for some distance along the inner, posterior face of arch IV (*labm IV*, Text-fig. 5, p. 141).

Urodeles.

Edgeworth (1935, p. 131) says: 'Four Levatores arcuum are present in larvae. Levator I arises from the auditory capsule or the dorsal fascia, Levatores II, III, and IV from the dorsal fascia. They are inserted into the dorsal ends of the kerato-

branchialia.' All four arise from the back of the capsule in *Triton alpestris* (Litzelmann, 1923, fig. 9).

Modern-type Anura.

The levatores (of which no. IV in all and nos. I to IV in the Pipidae, are undivided levators+constrictors) take origin far forward on the wall of the auditory capsule (*Pipa* and *Discoglossus*), or on this and the quadrate (*Xenopus*, *Bufo*, and *Rana*), or all on the quadrate (*Pelobates*; Edgeworth, p. 133 and personal observations).

Thus *Ascaphus* shares the more primitive arrangement of the Urodeles, with only a slight change towards the advanced anuran type. *Discoglossus* is also more primitive than most other frogs so far described.

(e) The Constrictores Branchiales Muscles.

Ascaphus.

Constrictor I is a muscle of about five fibres attached to the ventro-medial end of the under side of arch II (*cbm* I, Text-figs. 2 and 3). It passes laterally and comes to underlie and run with the 1st afferent artery under arch I (Text-figs. 3 and 4); it then loops backwards and upwards to its dorsal attachment on the extreme postero-dorsal tip of arch I, where this is in fusion with arch II at the terminal commissure. It is not in continuity with levator I.

Constrictor II. The size and the relations of C. II to arches II and III are just those of C. I to arches I and II (Text-figs. 3 to 5).

Constrictor III is a muscle of one fibre, lying in a loop under the arteries of arch III. It ends freely against the walls of the arteries both above and below, is not inserted on any cartilages and does not reach so far dorsally or ventrally as Cs. I or II. See Text-fig. 5, p. 181.

There is no separate constrictor IV, though levator+constrictor IV reaches relatively further ventrally than the levators do in arches I to III. There is no constrictor V.

Urodeles.

There are no constrictor muscles in Urodeles, unless they are represented by the small, dorsally lying depressores branchiarum muscles to the external gills; such occur in arches I to III, like the anuran muscles.

In this character, then, *Ascaphus* and the other frogs are unlike the Urodeles.

Discoglossus.

Constrictor I is just as in *Ascaphus*. Cs. II and III are both attached to the incipient processus branchialis from the base of arch III. As in *Ascaphus*, C. III ends against the efferent blood-vessel at its dorsal end and is not inserted on the cartilage arch; it is made up of two fibres only. C. II is attached dorsally to arch II.

Rana.

Cs. I and II are attached to the complete branchial process ventrally. C. III ends below against the blood-vessel and not against the cartilage, but it is attached to arch III dorsally.

(f) The Subarcuales Recti Muscles.

Ascaphus.

Subarcualis rectus I arises on the under side of arch I (*sarm I*, Text-fig. 1), and runs forwards and inwards, to be inserted on the posterior edge of the ceratohyal, just laterally to its posterior process (fig. 12, Pl. 10).

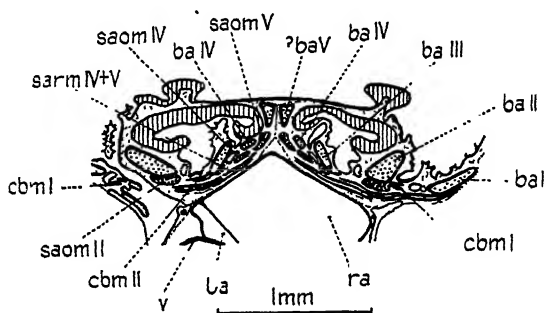
Subarcuales recti IV and ? V. There is no S. rectus II. Small bundles of fibres arise from the under sides of spiculum IV (? Vth gill arch) and arch IV (*sarm V* and *IV*, Text-fig. 4). The two bundles run forwards and outwards and combine their fibres into a larger, single bundle which passes under arches III and II without receiving fibres from them,¹ to be inserted on arch I, at a point just behind the origin of S. rectus I (Text-figs. 3 and 2).

¹ From transverse sections it is not easy to be absolutely certain that no fibres are added to the muscle from arch III; certainly none are added from arch II.

These are presumably segmental elements of branchial segments V and IV, combined anteriorly into a single muscle, inserted on arch I.

Urodeles.

S. rectus I is present as in *Ascaphus*. There is, of course, no Vth cartilage arch in the Urodeles. The remaining *S. recti* muscles (*S. rectus IV* only, of Edgeworth) have different relations from those of *Ascaphus*, although, undoubtedly,



TEXT-FIG. 3.

Section 12-1-7, through line xx in fig. 21, Pl. 14. For key to lettering see p. 181.

from their position and relations, they are the homologues of the anuran muscles. Edgeworth calls them all *S. rectus IV* and Eaton (1936) uses this same nomenclature. Eaton writes, on p. 65: 'The commonest and most primitive condition' in the Urodeles 'is for this muscle to originate on the fourth arch and insert on each of the three in front of it, continuing, however, as a single muscle. In *Amblystoma* larvae and those of the *Salamandridae* this condition is maintained.' I have checked the accuracy of this statement on both transverse and longitudinal sections of *Amblystoma*, *Salamandra*, and *Necturus*, and there is no doubt that all the fibres are attached to the IVth arch and that they are then shared out in smaller slips which are attached to arches III, II, and I. This arrangement differs from that in *Ascaphus* and

Discoglossus, where the fibres are attached in smaller bundles on the posterior arches and join to a single larger bundle, all of whose fibres are attached to the 1st arch.

Discoglossus.

S. rectus I is as in *Ascaphus*. There is no S. rectus II, or V. There is, however, a S. rectus III which is probably absent from *Ascaphus*. S. recti III and IV arise on the under sides of arches III and IV and, mingling their fibres, are inserted as one bundle on arch I, like the Vth and IVth muscles of *Ascaphus*.

Rana (in the fully formed larva).

S. rectus I passes from arch I to the ceratohyal. ? S. rectus II passes from the IInd arch and the anterior face of the branchial process to the ceratohyal, joining with S. rectus I in front. There is no S. rectus III, whilst S. rectus IV runs from the IVth arch to the posterior face of the branchial process.

(g) The Subarcuales Obliqui Muscles.

Ascaphus.

Small bundles of fibres arise on the under sides of (1) spiculum IV (= ? arch V), (2) arch IV, (3) arch III, and (4) a larger bundle on arch II (*saom* I'-II, Text-figs. 4, 3, and 2). Bundles 1 and 2 fuse together on passing forwards (Text-fig. 2, right side) and later fuse again with bundle 3 (Text-fig. 1); the resulting two bundles pass forwards and inwards (ventrally to the arterial arches, but dorsally to the subarcuales recti muscles mentioned above) and are inserted, one behind the other, on the outer side of the urobranchial prong near its tip (Text-fig. 1).

Ascaphus thus possesses four pairs of subarcuales obliqui muscles, one pair in each of branchial segments II to V.

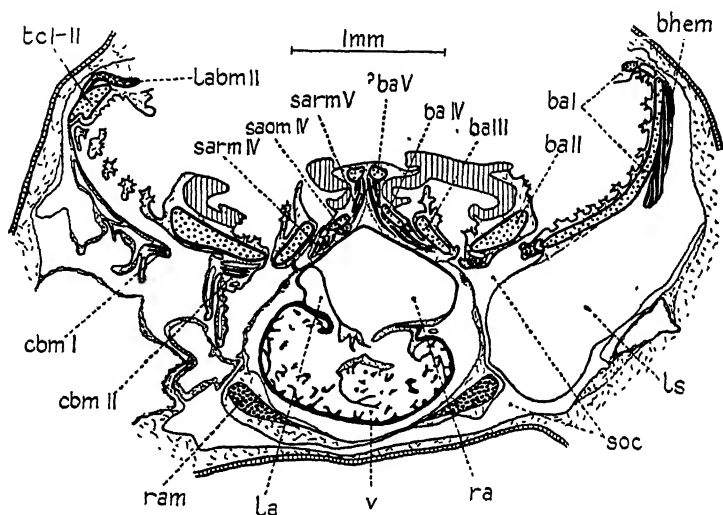
Urodeles.

There are only two pairs of oblique muscles in *Urodeles*; these arise on the under sides of ceratobranchials II and III

and pass forwards and inwards, fuse together and insert by single tendons, either on the urobranchial prongs, or on the sheaths of the recti cervicis muscles in this region.

Modern-type Anura.

In the Anura, including the Discoglossidae, there is but a



TEXT-FIG. 4.

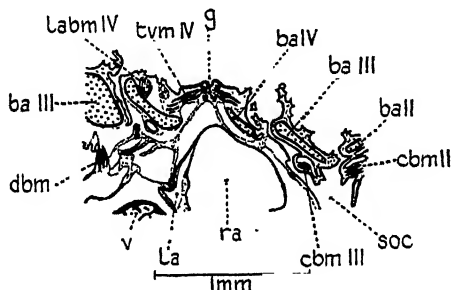
Section 12-3-1, through line *xy* in fig. 21, Pl. 14. For key to lettering see p. 181.

single pair of oblique muscles, present in branchial segment II, corresponding to the largest of the four pairs in *Ascaphus*. Arising on the under sides of the IIInd arches, the muscles pass inwards and slightly forwards to meet one another in a median raphe which is attached loosely by ligaments to the posterior tip of the undivided urobranchial keel of the basibranchial copula. This keel, it may be noticed, is better developed and is deeper in *Discoglossus* than in other frogs.

Edgeworth (1935) gives just such an account of this muscle pair on pp. 161 and 162 of his monograph, yet he calls each muscle a 'transversus ventralis II'. He thus fails to recognize the

obvious homology with the oblique muscles of Urodeles (and *Ascaphus*), his failure probably being due to his theoretical conceptions of the primitive, ancestral content of muscles in any one branchial segment (see discussion on p. 142).

In respect, therefore, of the subarcuales obliqui muscles, *Ascaphus* is the most primitive of the living tetrapods. The Urodeles retain a half



TEXT-FIG. 5.

Section 12-4-8, through line zz in fig. 21, Pl. 14. For key to lettering see p. 181.

and the modern-type frogs a quarter of this ancient inheritance.

(h) The Transversi ventrales Muscles.

Ascaphus.

The only transversus ventralis muscle present in *Ascaphus* is that in branchial segment IV. (The transversus ventralis II, of Edgeworth's nomenclature for the Anura, has been described above as part of the subarcuales obliqui system.) The fourth transverse muscle arises in mesenchyme close to the inner, posterior border of the IVth arch, as a poorly developed sheet of fibres which passes inwards and upwards, to meet its fellow in a median raphe in the middle line, just in front of the very small glottis; further back, the glottis divides the two muscles (Text-fig. 5, *tvm IV*).

Modern-type Anura.

Such a muscle pair begins to develop in young, modern-type

larvae, but disappears again very early, even in the Discoglossidae.

Urodeles.

The muscle pair is represented in Urodeles by a very large sheet of muscle lying between the IV arches and even spreading to other attachments.

The retention of a pair of transversus ventralis IV muscles into the well-developed larva is a further character which *Ascaphus* alone shares with the Urodeles. (A dilator laryngis, with which this transversus ventralis IV might be confused, is also present in *Ascaphus*.)

(i) Discussion.

On page 162 of his monograph Edgeworth writes: 'The primitive condition of the Subarcuales recti was probably one in which each was attached to the branchial bar of its segment of origin and extended forwards to the next anterior bar, and that of the Transversi ventrales one in which each passed to a median raphe. Dipnoi, Teleostomi, and Amphibia probably had a Subarcualis rectus and a Transversus ventralis in each branchial segment.' On p. 156 he writes: 'In Urodela Subarcualis rectus I passes from the 1st branchial bar to the hyoid bar. The other, more posterior, muscles are modified in association with the development of the urobranchial outgrowth of the basibranchiale. In genera with four branchial bars Subarcuales II and III diverge inwards and unite in a common tendon which is inserted into the urobranchiale, forming Subarcuales obliqui II and III. Subarcualis rectus IV grows forwards beyond the next bar . . . in three fasciculi inserted into the 1st, IIInd, and IIIrd bars.' Clearly, then, Edgeworth's view is that a branchial segment may contain a Transversus ventralis and a Subarcualis muscle and this latter can be either in the 'rectus' or in the 'obliquus' form, but presumably both subarcualis types cannot be present at once. He fits the facts to this theory by assuming that his 'Subarcualis rectus IV' muscle of the Urodeles is the product only of the IVth muscle plate, in spite of its three fasciculi to the IIIrd, IIInd, and 1st arches. Eaton (1936),

however, writes on p. 65: 'The muscle is built embryonically out of part of the ventral material of the second, third, and fourth branchial muscle plates', and Druner, to quote from Edgeworth, p. 157, found that in the Urodeles there is 'an additional innervation of the anterior part of Subarcualis rectus IV corresponding to the segments into which it grows'; this innervation is from the branchial branches of the vagus nerve. These facts, then, surely indicate that the so-called Subarcualis rectus IV is really a combined form of Subarcuales recti IV, III, and II, where possibly the IVth has grown forwards to the 1st arch and III and II have grown backwards to the IVth. If, however, we adhere to Edgeworth's idea that a branchial segment cannot contain both a *S. obliquus* and a *S. rectus* at the same time, we are immediately in difficulties in the case of the Urodeles, for they certainly contain oblique muscles in segments II and III, and we are now suggesting that these segments also possess recti muscles as well. Edgeworth himself overcame this difficulty by calling this rectus complex simply '*S. rectus IV*'. There was a further difficulty for him in the case of the frogs. He found that the advanced frogs possessed simple *S. recti* muscles in each of segments I to IV in early development; but they also possessed, on each side in segment II, the muscle which has been called *S. obliquus II* on p. 140 of this paper. Edgeworth was compelled to overlook the clear homology which this muscle shows to the IInd *S. oblique* muscle of the Urodeles and was forced to call it a *Transversus ventralis II*. With these reservations Edgeworth's system covered all the animals described up to 1935.

A knowledge of the muscles of *Ascaphus* introduces a new difficulty for Edgeworth's theory. In this animal there are *S. obliqui* muscles in each of branchial segments II, III, IV, and ? V, and there are *S. recti* muscles in segments I, IV, and ? V. This difficulty could be resolved by extending Edgeworth's system of calling the *S. obliqui* muscles of the frogs the *Transversi ventrales* muscles and disregarding their obvious similarity to the Urodele *S. obliqui* muscles, if only there were no persistent *Transversus ventralis* muscle in segment IV. This IVth segment, however, contains at one and the same time a *S. rectus* pair,

a S. oblique pair, and a T. ventralis pair. Consequently the S. obliqui muscles of *Ascaphus* cannot be disposed of as T. ventrales muscles, but must remain as S. obliqui. This means, then, that in segments IV and ? V there are both S. recti and S. obliqui muscles. A realization of this possibility allows us also to call Edgeworth's 'T. ventralis II' of the modern-type frogs the S. obliquus II.

Certain changes must, thus, now be made. (1) We must be willing to face the probability that a primitive branchial segment could contain simultaneously a S. rectus, a S. obliquus, and a Transversus ventralis muscle pair. (2) In future the term 'Transversus ventralis II' must be abandoned in favour of 'S. obliquus II' in the Anura. (3) The term 'Subarcualis rectus IV' in the Urodela should probably be altered to 'S. recti IV, III, and II', especially as the ? Vth S. rectus muscle in *Ascaphus* and the IIIrd S. rectus in *Discoglossus* have no connexion with arch IV at all.

Clearly, then, this whole subject requires further careful research into the origins of these various muscles in the early developmental stages of Urodeles, *Ascaphus*, and the modern-type frogs. It would also be most valuable to investigate the arrangement in *Liopelma*. The attachments of the posterior S. recti muscles are different in *Ascaphus* from those in the Urodeles and it is necessary to find out how these differences arise in development.

(j) The Diaphragmato-branchialis Muscles.

In *Pelobates* Schulze (1892) described a small muscle running from the posterior face of the IVth arch, near its top, to the postero-lateral side of the pericardium ('diaphragm') just in front of the glenoid region of the pectoral girdle. This muscle is also present in *Ascaphus* (*dbm*, Text-fig. 5), *Discoglossus*, and *Rana*; yet, in the well-formed larvae of each of these genera, it is attached to the IIIrd arch. near the terminal commissure with the IVth and not to the IVth arch as Schulze held (personal observation).

By description, this muscle would seem to be the homologue of the omo-arcualis muscle of certain Urodeles.

6. THE HYPOBRANCHIAL SPINAL MUSCLES OF ASCAPHUS.

(a) The Geniohyoideus Muscles.

The geniohyoideus muscles, in *Ascaphus*, pass from the postero-median ends of the anterior jaw cartilages to the outer edges of the basibranchial copula and the hypobranchial plates where the 1st and 2nd arches pass into them; parts of the muscles are attached on the bases of the 1st arches themselves. These relations are more or less those of the other *Anura*. On the right side of this particular specimen, however, (but not on the left) two fibres detach themselves from the inner border of the muscle (unlabelled, figs. 9, 10, Pl. 9; figs. 11, 12, Pl. 10) and run an independent, backward course, resting on the interhyoideus muscle, to become attached, with the tip of the rectus cervicis muscle, to the outer side of the right urobranchial prong, near its tip. In this small point this specimen imitates the *Urodeles* whose geniohyoideus muscles are attached along the front border of the forked end of the urobranchial prong (e.g. *Salamandra*) or on the urobranchiale and the recti cervicis muscles (e.g. *Amblystoma*).

(b) The Recti Cervicis Muscles.

In *Ascaphus* each rectus cervicis muscle is attached in front on the ventro-lateral tip of the urobranchial prong (*rcm*, Text-fig. 1) and passes sharply downwards and backwards, round the antero-lateral wall of the pericardium, to be attached, behind, on the ventral membranous sheets below the heart (Text-fig. 2). The muscles do not reach back to the girdle. The lower, front border of each muscle is made up of thin, young fibres (*rcsm*, Text-fig. 1) which may represent a superficialis bundle. The muscles are very much smaller than in the *Urodeles*, and do not fill up the space above the urobranchiale and below the hypobranchial plates. They are also smaller than in the more advanced frogs, including *Discoglossus*. The attachment of the muscles to the urobranchiale is a character which *Ascaphus* shares with the *Urodeles*, although in the *Urodeles* the huge muscles have

more extensive attachments spreading beyond the urobranchiale to the basibranchiale and to one or two hypobranchialia.

In the other Anura, the muscle is attached to the bases of the IIrd and IIIrd arches (in *Discoglossus*), or to the base of the IIIrd arch and the processus branchialis (in *Rana*), whilst (in both genera) some fibres start from mesenchyme near the subarcualis rectus IV muscle. The fibres are not associated with the small urobranchial keel of the basibranchial copula.

7. DISCUSSION.

(a) The Position of the Splanchnic Structures of *Ascaphus* and other Frog Larvae.

The foregoing descriptions make it clear that the splanchnic structures of the larval *Ascaphus* have an arrangement rather dissimilar to that found in any other of the surviving frogs. For this reason the term 'modern-type' anuran has been used throughout this paper to sum up all the other frogs taken together, in contrast to the more primitive type shown by *Ascaphus* (and presumably by *Liopelma*, though this form has not been described in its larval condition). The smaller differences between the larvae of the modern-type frogs are insignificant when measured against the differences which any one of them shows when compared with *Ascaphus*. The head structures of all modern-type frogs show but slight variations from a single basic plan, but the structures of *Ascaphus* cannot be fitted to this plan without considerable reservation.

Following Noble (1924, 1927, 1931, &c.) and de Villiers (1934), it is customary now to look upon the Liopelmidæ, which is the family containing *Ascaphus*, as the most primitive family of living frogs, and the facts set out in this paper give strong support to this view. Consequently, a detailed knowledge of the anatomy of *Ascaphus*, throughout its life history, may be expected to be of first-rate importance to the understanding of the origins and relations of the isolated and somewhat anomalous group, Anura, with its peculiar larval specializations.

Luther (1914, p. 94 et seq.) attributed these larval anuran specializations to a single cause. Edgeworth (1935) is in agree-

ment with Luther's thesis. and writes as follows (p. 237): 'Luther was of the opinion, and I think rightly, that the causes are to be found in the great length of the intestine—an adaptation to vegetable food—which brought about a ballooning of the peritoneal cavity and its forward extension. This produced a forward migration of all organs lying in front of the peritoneal cavity and below the chondrocranium. . . . The influence of the elongation of the intestine is probably not direct and mechanical but hereditarily fixed.' Whether these writers have picked on the right cause or not, and there seems to be no good reason to dispute their view, there can be no doubt that such an extensive forward movement of all the splanchnic structures has taken place in phylogeny, and it is this forward movement which has given to the larval, modern-type anuran head its very peculiar arrangement. What follows on p. 168 of this paper makes it clear too that even the possession of the peculiar muscular process on the quadrate by larvae of modern-type frogs is a direct result of this general forward movement, though perhaps this had not been realized before.

If a Urodele is taken as a standard of the more typical Vertebrate for the purposes of comparison, it will be seen that the following structures in the modern-type frogs have all moved forwards from their ancestral position:

- (1) The trabecular horns (and their probable derivatives, the supra-rostral system).
- (2) The nasal sac and its surrounding capsule.
- (3) The lower jaw system.
- (4) The whole quadrate bar, particularly:
 - (a) the articular region;
 - (b) the commissura quadrato-cranialis anterior (= the 'anterior basal process', see p. 154);
 - (c) the pterygoid process;
 - (d) the muscular process (= the specialized anterior end of the ancestral otic process).
- (5) The position of the mouth opening.
- (6) The columella system (and annulus) of the ear apparatus.
- (7) The hyobranchial apparatus as a whole and the centre of each arch in particular.

(8) The Eustachian tube (= hyoid gill pouch) and subsequent gill slits.

(9) The mandibular, hyoid, and branchial muscles and the hypobranchial spinal muscles, their nerves and blood-vessels.

(10) The heart and arterial arches.

(11) Certain bone rudiments, particularly of the following bones:

- (a) pterygoid;
- (b) quadratojugal;
- (c) premaxillary;
- (d) maxillary.

In every one of these characters (except no. 6, and nos. 8 (in part) and 11) the larval head of *Ascaphus* is certainly less modified from the primitive vertebrate plan than are the larval heads of the modern-type frogs. There is no evidence in relation to characters 6 and 8 (in part), since the adult *Ascaphus* has no middle ear cavity or tube, nor columella apparatus or annular cartilage (de Villiers, 1934). I have found no certain trace of any of these characters in the single larval specimen studied, but see p. 173. Further, this particular specimen is too young to show traces of the rudiments of the squamosal, premaxillary, and maxillary bones; however, since the cartilages to which these bones are related lie further back in *Ascaphus*, the bones themselves must also lie more posteriorly when they come to be formed, in later stages. The pterygoid rudiment (= the quadrato-ethmoidal ligament) is certainly farther back, and this is true also of the 'trabecular-quadrato ligament', from part of which the quadratojugal bone is ossified in *Rana*; however, no quadratojugal bone is formed in *Ascaphus* (Noble, 1931, and de Villiers, 1943).

It would thus seem that *Ascaphus* is a persistently primitive frog which supplies a most valuable link in the history of the evolution of the Anura.

- (b) Is the Cranial Ground-plan of *Ascaphus*
really Primitive?

It is well at this point to face a legitimate criticism. The tadpoles of *Ascaphus* live in fast-flowing water and in this

connexion possess a powerful sucker formed from the larval 'lips'; they also employ a peculiar method of progression whilst they are attached to the substrate and a specialized method of particle feeding (Noble, 1927). Such adaptations might be expected to be correlated with considerable changes in the anatomical ground-plan of the larval head. It could be argued that, if Luther is right in believing that a single cause can have modified a large number of structures away from the primitive vertebrate plan, some other single cause might be found responsible for a general reversal of these modifications. It could be suggested that perhaps the requirements of a sucker system had secondarily forced back all the splanchnic structures to their primitive position. If this were true, the anatomy of the larval *Ascaphus* would only simulate the primitive condition, whilst actually being secondary in nature, and this animal would not be a true guide to an understanding of the evolution of the advanced frogs.

A number of small points from the anatomy of *Ascaphus* could be brought forward in apparent support of this view. For example: (1) the medial piece of the supra-rostral system seems to be too far back to be just a simple downturning of the primitive trabecular horns; (2) the membraneous 'posterior narial tube' perhaps suggests a backward migration; (3) the posterior origins of the adductor jaw muscles from the auditory capsule could be interpreted on this theory; (4) the lower jaw lies transversely across the throat and does not take the form of a U with its anterior end well forward in the snout, in spite of the fact that its articular facets are extraordinarily far back for a frog; (5) the absence of an independent levator mandibulae anterior (pterygoid) muscle may be suspicious, and could be explained as due to the forcing back of a more anterior commissure; (6) the presence of a pre-oral buccal cavity would agree with this view; (7) and finally, Sæve-Söderbergh's idea of the nature of the anuran commissure would fall into line with this possible theory.

It seems to me to be likely that a slight backward movement of structures has taken place in the front of the snout in connexion with the consolidation of the supra-rostral system,

but, for the reasons set out below, I am convinced that there has been no general backward movement of all the splanchnic structures in the evolution of the skull of *Ascaphus*. It is worth noticing here, however, that there probably are characters in *Ascaphus* which are correlated with the sucker mechanism and the mode of progression. Such probably are: (1) the general heavy build of the head cartilages; (2) the great strength of the adductor jaw muscles; (3) the exaggerated autostyly of the palatoquadrate with its extensive commissura and with the additional fusion of the auditory capsule to the posterior border of the ascending process; (4) the extensive and rigid fusion of the central part of the supra-rostral system to the ethmoid region of the skull; (5) the great antero-posterior width of the posterior jaw cartilages, with their posterior spurs; and (6) the consolidation of the whole hyobranchial apparatus and the width of the individual arches.

Against the view that the cranial plan of *Ascaphus* has been derived from the modern-type plan by a secondary return of its structures to an apparently more primitive posterior position, several points may be brought forward. (1) From evidence largely unconnected with its head structures, *Ascaphus* has been placed with *Liopelma* in the most primitive family of living frogs. The order Anura is marked off from the other vertebrate groups, among other things, by the larval specializations of its members. These specializations extend particularly to the splanchnic head structures, as shown above, and must have been the result of very great evolutionary changes. It would be surprising, therefore, if the most primitive living member of the order failed to show some trace of the ancestral plan. It would be more surprising still if, in fact, that imposing array of apparently primitive characters which *Ascaphus* admittedly possesses was due to some secondary cause and was not a simple retention of the ancestral inheritance. In view of the other evidence to be given below, the general contention that *Ascaphus* is persistently primitive seems to me to hold good in spite of the fact that such 'link-animals' usually retain a footing in the modern world only at the expense of specialized modifications

to fit some particular niche. The modifications which have allowed *Ascaphus* to survive are perhaps those related to the sucker apparatus; this is better developed than in any other frog, and its evolution has permitted *Ascaphus* to colonize the fast-flowing streams in which its tadpoles develop.

It thus becomes important to disentangle the primitive from the specialized in *Ascaphus*. This perhaps can best be done by extending anatomical studies to embrace *Liopelma* whose larval adaptations are those to suit a terrestrial development (Archey, 1922) and not a development in fast streams. Those characters, then, which *Ascaphus* shares with *Liopelma* might be accepted as part of their common ancestral inheritance. But even this test may prove to be not wholly critical, since both these Liopelmid frogs are without a middle ear apparatus (de Villiers, 1934, and Wagner, 1934, 1 and 2), although such was, of course, present in the ancestor and even in so close a relative as *Protobatrachus* (Piveteau, 1937). Whether this apparatus was lost by the more recent common ancestor of the Liopelmidae, or whether each of the modern genera has lost it separately cannot be known at present. The same type of difficulty may therefore be expected to concern other characters shown commonly by the two frogs, in view of the great Amphibian tendency to parallel evolution which has been brought out by Watson's researches. Investigations of the larval modifications of other frog tadpoles adapted to fast water may give help in this important problem. Unfortunately, I have not had access to material either of *Liopelma*, nor of any of the mountain-brook tadpoles.

(2) In many of those characters in which *Ascaphus* differs from the modern-type frogs it approaches more nearly to the Urodeles; see the list on p. 175. This is significant and militates against the contentions put forward by certain Scandinavian workers (Holmgren, 1934, and S  ve-S  derbergh, 1934 and 1935) that the Anura and the Urodela are separately derived from the Fish. A knowledge of the Urodele head is of great importance in the interpretation of the head of *Ascaphus*.

(3) The family Discoglossidae is the group of frogs which Noble believes to be the next most primitive after the

Liopelmidae. My own unpublished studies of the larval cranial structures of *Discoglossus pictus* show that this frog is, in many small points, intermediate between *Ascaphus* and such a modern-type frog as *Rana temporaria*. Now the tadpoles of *Discoglossus* live in pond water and are not tainted with the specializations connected with a sucker mechanism, yet their cranial structures are best explained by assuming that there is some retention of the plan shown in *Ascaphus*. This would seem to prove that *Ascaphus* is truly primitive in its head form and not merely secondary. A list of the similarities of *Ascaphus* and *Discoglossus* is given on p. 177.

(4) The hyo-branchial apparatus, the jaw and the branchial muscles may be picked out for special mention. In respect of these structures *Ascaphus* is almost more like a Urodele than it is like the modern-type frogs, whilst in some points it is perhaps even more primitive than the Urodeles. This could hardly be so if *Ascaphus* had passed through the modern-type evolutionary stage, to return, by later specialization, to a secondary state of apparent primitiveness.

(5) Finally, it is perhaps worth noticing that a forecast was made in a previous paper (Pusey, 1938) of many of the characters to be expected in the larva of any ancestral frog. This forecast was based on a study of the tadpoles of *Rana* and on the published accounts of the larval and adult skulls of *Alytes* and on the adult skulls only of *Ascaphus* and *Liopelma*. At that time nothing pertinent was known of the larval structures of *Ascaphus*. Yet these structures, as set out here, bear out that forecast in a remarkable way and in a way hardly possible if they are secondary and not primitive.

From the above evidence, therefore, it will be taken for granted in what follows that larval *Ascaphus* supplies a link in the chain of evolutionary stages leading from the ancestral to the specialized larva of the modern-type frog, of which, for convenience, *Rana temporaria* may be taken as an example.

(c) Extrapolation to the Larval Ancestor
of the Frogs.

Speaking figuratively, our present knowledge of anuran arvae supplies us with three main points on the 'evolutionary graph' which should extend from the primitive pre-anuran ancestor at the one end to the modern-type anuran, such as *Rana*, at the other. These three points are not evenly spaced along the graph. (1) The point supplied by *Rana* (and the other modern-type genera) lies at the extreme specialized end of the graph. (2) The point '*Discoglossus*' lies close to it, but a little nearer to the ancestral end; *Bombina* and *Alytes* would lie close to *Discoglossus*, but on the side of *Rana*. (3) Half-way down the graph lies *Ascaphus*, parted from *Discoglossus* and *Rana* by a big gap. The point representing the ancestral pre-anuran would lie far beyond *Ascaphus* on the side away from *Rana*. From our present knowledge we are unable to fix this last point with any certainty, especially from the larval point of view, although the adult anatomy of the pre-anuran ancestor is becoming clearer from the recent work of Piveteau (1937) and Watson (1940); perhaps the anatomy both of the larval and adult *Urodele* may be taken as a generalized guide.

If we think in this graphical way of the smooth set of changes which must have taken place in phylogeny, modifying the ancestral pre-anuran plan to the plan of the modern-type frogs, we can think also of extrapolation. Our curve can be drawn backwards, with fair certainty, through *Rana*, *Discoglossus*, and *Ascaphus*, and this is sufficient to give its general course. By extrapolation into past time we can build up for ourselves an idea of the ancestral organization, by reversing the changes which have taken place along the known part of the curve. We can thus obtain a fairly exact idea, also, of the homologies of the anatomical parts present in the modern-type frog, and this is largely the purpose of the following analysis.

The difficulty in understanding larval anuran head structures

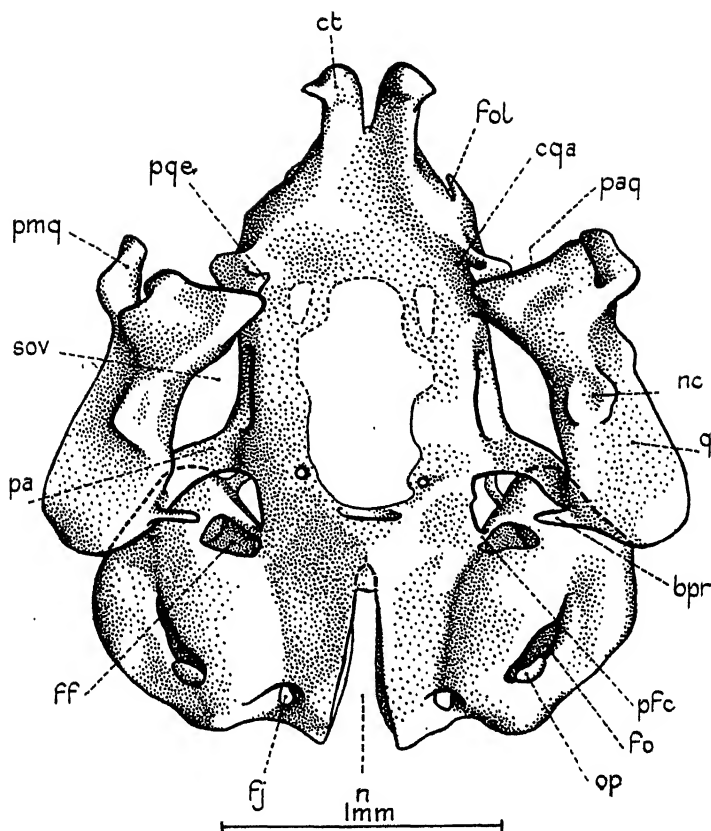
has always lain with the interpretation of the parts of the palatoquadrate. The supra-rostral system also supplies difficulties. Most other structures, however, though modified, are identifiable in terms of, say, Urodele structure, and therefore call for less attention.

(d) The 'Anterior Basal Process' (= Gaupp's Commissura) and the 'Posterior Basal Process.'

The two parts of the modern-type anuran palatoquadrate system which have been the most difficult of interpretation are the commissura quadrato-cranialis anterior (of Gaupp) and the pseudobasal process (of de Beer, = basal process of Gaupp). The issue is further complicated (or perhaps simplified) by an additional process—the 'posterior basal process' of this paper—which is present in *Ascaphus*, and which still just survives in *Discoglossus*, but which is totally absent from all other modern-type frogs, including *Alytes* and *Bombina*, of the family *Discoglossidae*.

It will be unnecessary to enter into any discussion of the nature of the pseudobasal process of modern frogs until sections have been cut of the metamorphic stages of *Ascaphus*, for the larva of *Ascaphus* throws no new light on the problem, since the process is not yet formed at the stage here studied. But, in connexion with this structure, it may be pointed out here that the larval *Ascaphus* possesses two processes, each of which, in part, represents the typical Craniate basal process (see below). Neither of these, however, is the same as the pseudobasal process of modern frogs, so that *Ascaphus* supports the contention of a previous paper (Pusey, 1938), that, whatever may be the nature of the pseudobasal process, it is not the quadrate basal process, as Gaupp and others have believed.

In the modern-type frog tadpoles, the commissura is a relatively narrow band of cartilage extending from the level of the olfactory foramen in front, to a position mid-way between this and the optic foramen behind (see Pusey, 1938, fig. 8, and particularly Parker's papers, 1876 and 1881, and also Text-fig. 7 E of this paper). Between its posterior edge and the front of



TEXT-FIG. 6.

Discoglossus pictus. Reconstruction of the neurocranium and palatoquadrate of a young larva in ventral view, to show the presence of a small 'posterior basal process', *bpr*. Overall length of larva 15.2 mm. and length from anus to tail tip, 8.7 mm.; the supra-rostral and lower jaw systems have been removed. For the key to the lettering see p. 181.

the auditory capsule lies the extensive subocular vacuity which is such a striking feature of the modern-type tadpole's skull. In *Ascaphus*, however, the anterior border of the commissure begins where the posterior border of the modern tadpole's commissure ends, whilst the posterior border lies far back under the

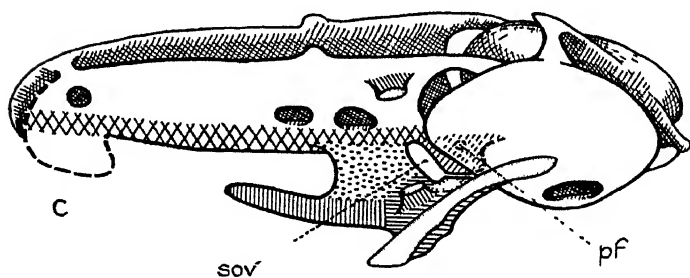
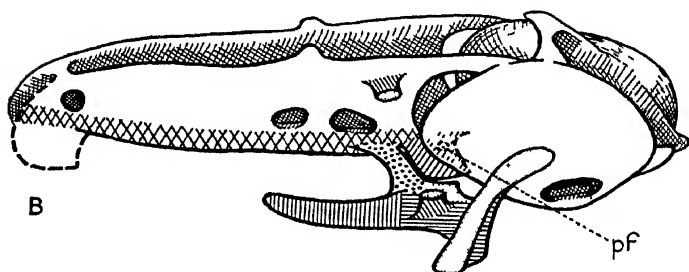
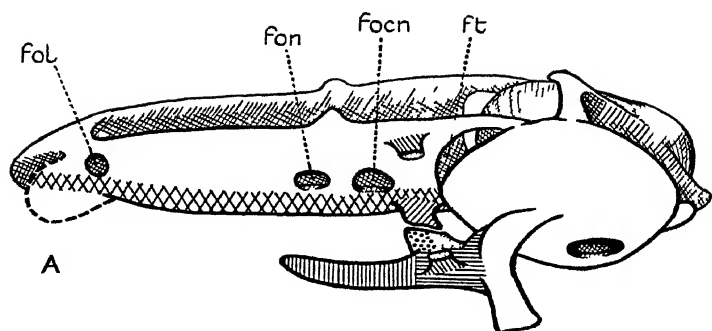
ascending process and close in front of the auditory capsule. Thus the commissure in *Ascaphus* is a much wider band and lies much farther back in the head. As a result, there is no subocular vacuity visible in dorsal view; this space is only a small pear-shaped opening lying just in front of the auditory capsule; the relations are clearly shown in fig. 17, Pl. 12, and Text-fig. 7 D. If, then, in the ancestor of *Ascaphus*, the commissure was still farther back, as our extrapolation idea would suggest, we must envisage the possibility that the subocular vacuity was then entirely absent and that the hinder border of the commissure abutted against the whole front border of the basitrabecular process, which in *Ascaphus* is incorporated in the floor of the auditory capsule. A result of this would be that the commissure would have formed one continuous band with what has been called the posterior basal process (*bpr*, fig. 17, Pl. 12, and Text-fig. 7 C and B), and this single cartilage boss so formed would have projected backwards and inwards from the body of the quadrate, to articulate with the basitrabecular process. This boss would then have been the equivalent of the basal process of the Urodeles and other Craniates, except that it would have been fused to the trabecula in front of the basitrabecular process, as well as simply abutting against this process behind. It is from this line of argument that I have been led to call the commissure the 'anterior basal process' and the process marked in the figures as *bpr* the 'posterior basal process', for I consider these two structures to be the products of the division of one, ancestral, basal process (Text-fig. 7 A). This argument was previously set out in another paper (Pusey, 1938), but at that time it appeared that the commissure was the entire basal process shifted forwards, for the very small posterior basal process of *Discoglossus* had only been found after the text was written (see footnote on p. 530 of that paper), and, of course, nothing was then known of the large posterior basal process of *Ascaphus*.

Watson (1940) has shown that the position of the basitrabecular process (as indicated by the bony basipterygoid process) has shifted backwards in the phylogeny of the frogs'

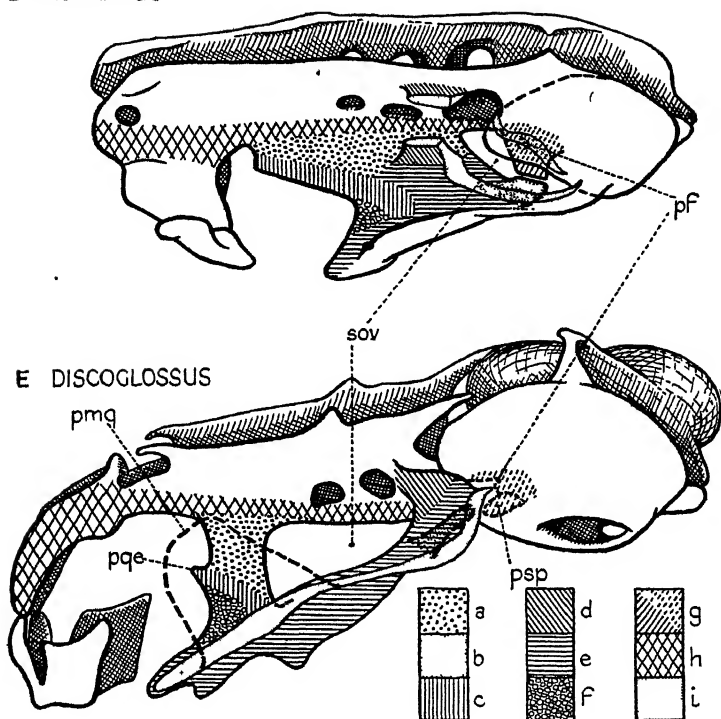
ancestors. In *Eugyrinus wildi*, of the Lower Coal Measures of Lancashire (his fig. 13), it lay in front of the auditory capsule and apparently independently of its floor. In *Miobatrachus romeri*, of the Coal Measures of Mazon Creek, Illinois (his fig. 4), it appears to have been a semi-independent part of the capsular floor, still, however, partially separated from it by a notch. In *Protobatrachus massinoti*, of the Basal Trias of Madagascar (his fig. 19), it is the effective floor of the capsule, as in *Ascaphus*. See also Watson's comparative sketches shown in his figs. 17 and 21. These comparisons bring out the fact that, in addition to shifting backwards, the basipterygoid process has also spread more and more laterally.

(e) The Postulated Ancestral Larva and its
Evolution to the Modern-type Tadpole.

With a knowledge of the tadpole of *Ascaphus*, coupled with the findings of Watson's recent researches, we can hazard a guess at the larval conditions found in the ancestors of the frogs. It is obvious that in the earliest forms, before larval specialization had been evolved, a single basal process from the palatoquadrate met a basitrabecular process which projected from the cranial base some way in front of the auditory capsule and quite independently of it (Text-fig. 7 A). With the onset of larval specialization in phylogeny two things seem to have taken place. (1) The basal process gained a new and increasing fusion directly to the trabecula, in front of the basitrabecular process; this would have been the incipient commissure (Text-fig. 7 B). (2) The basitrabecular process developed further and further back in each ontogeny, and yet always retained an articulation, or fusion, with the posterior corner of the quadrate basal process (posterior basal process) (Text-fig. 7 A to C). With increasing specialization these movements went in opposite directions, the one forwards and the other backwards. As a result, we may assume that the subocular vacuity opened up as a gap in the articulation between the now broad and divided basal process, on the outer side, and the retreating basitrabecular process on the inside (Text-fig. 7 C). These two forms of specialization would then have continued steadily.



D ASCAPHUS



TEXT-FIG. 7.

The suggested evolution of the larval anuran palatoquadrate system. A, B, and C are hypothetical ancestors representing larval stages of *Eugyrinus*, *Miobatrachus*, and *Protobatrachus* (at least in so far as the position of the basitrabecular process is concerned). D, larval *Ascaphus*, and E, larval *Discoglossus*. The ascending process in A, B, C, and D, parts of the otic process in B, C, and E, and the fusion of the ascending process with the tip of the auditory capsule in D have been cut away; cut surfaces are mechanically stippled.

a, 'anterior basal process' = commissura quadrato-cranialis anterior; b, 'posterior basal process'; c, pterygoid process; d, ascending process; e, body of quadrate; f, fusion of pars articularis with the under side of the pterygoid process; g, basitrabecular process and post-palatine commissure; h, trabecula; i, cut cartilage surfaces. For the key to the lettering see p. 181.

Let us take the commissura first. Judging from the condition in *Ascaphus*, we must assume that the anterior basal process (= commissura) rapidly increased its extent of fusion with the trabecula, so that finally the whole body of the quadrate and also the inner, upper border of the pterygoid process came to be fused to the whole length of the opposing trabecular cartilage (see fig. 17, Pl. 12, and Text-fig. 7 D). In this way the upper edge of the pterygoid process came to be masked in the commissure, but in *Ascaphus*, in contrast to the modern-type tadpole, its lower edge still indicates a lengthy process otherwise independent of the body of the quadrate. The lower edge of this pterygoid process, which is shod all along its under side with the rudiment of the pterygoid bone (= the quadratoethmoidal ligament), bears to the mouth cavity, the trabecula, and the body of the quadrate the typical relations which are found, for instance, in the fully formed *Urodele* larva.

With further specialization towards the modern-type tadpole condition we must assume that two changes took place in the commissure (Text-fig. 7 E). Firstly, that the position in which it is attached to the trabecula became more and more anterior in each ontogeny, until its front border reached the nasal sac. Secondly, that the antero-posterior width of the commissure decreased and its posterior edge came to lie very much farther forwards, in such a way that the fusion was now between the trabecula and the pterygoid process only, rather than, as in *Ascaphus*, between the trabecula and both the pterygoid process and the body of the quadrate (see Pusey, 1938, fig. 8). As a result of these two changes an increasing vacuity was opened up behind the commissure. *Ascaphus* has remained at the stage where the evolutionary processes are only just beginning, and where the subocular vacuity is insignificant and the whole of the commissure is broad and lies far back in the skull.

The importance of this whole argument is that it stresses the point that the commissure of the modern-type tadpole is a part of the basal process of the quadrate which primitively belongs to a part of the jaw system lying far back in the head. The commis-

sure is thus not a neomorph and is not a structure primitively belonging to the ethmoid region of the snout. This contention is but a slight modification of that set out on pp. 528 and 529 of a previous paper (Pusey, 1938), but it is in sharp contrast to the views held by Gaupp (1906), Edgeworth (1925), Säve-Söderbergh (1936), and Watson (1940), all of whom look on the commissure as essentially an ethmoidal structure. Gaupp's and Edgeworth's theories have been criticized in the paper mentioned, but Säve-Söderbergh's and Watson's views call for remark here.

(f) A Criticism of Säve-Söderbergh's Theory
of the Nature of the Commissura.

In interpreting the adult structures of the snout of *Lyrocephalus euri*, a triassic Stegocephalian, Säve-Söderbergh derived assistance from the larval cartilages of *Rana temporaria*, one of the most specialized of present-day frogs. As a result, he marks a postulated ethmoidal cartilage bridge between the front end of the quadrate pterygoid process and the ethmoidal region of the skull of his animal, as a commissura quadrato-cranialis anterior (his figs. 7 and 8). Had he been able to use the then unknown structures of the more primitive *Ascaphus* for comparison, this interpretation would hardly have been possible. To use a purely larval specialization of an admittedly specialized modern animal to interpret the structures in an adult of an ancient stock, particularly when the structure of the modern form is known to be lost at metamorphosis, seems to me but to court disaster. Watson, in his interpretation of the skull of *Branchiosaurus*, has accepted Säve-Söderbergh's lead (see Watson, 1940, fig. 23, and p. 226).

It may be well to collect together the evidence against this theory and to try to show finally that the commissure cannot be considered to be an ethmoidal structure.

(1) In *Ascaphus* the commissure is a mid-cranial and not an ethmoidal structure and, though its front border admittedly reaches up to the unusually posteriorly-placed internal nostril, its hind border is in the auditory region (Text-fig. 7 D).

(2) In *Lyrocephalus* and *Branchiosaurus* the

connexion in question is between the tip of the pterygoid process of the quadrate and the ethmoidal part of the trabecula. The whole of the pterygoid process and of the pterygoid bone lies behind it. In *Ascapus*, on the other hand, the commissure connects (1) the whole upper surface of the pterygoid process (and not just its tip) and (2) the greater part of the inner border of the body of the quadrate, to the trabecula, along their respective lengths, so that no part of the pterygoid process, or of the pterygoid bone rudiment underlying it, lies behind the commissure; in fact these structures lie rather in front of the commissure (Text-fig. 7 D).

(3) In modern-type frog larvae the tip of the future adult pterygoid process lies in front of the anterior border of the commissure, as the processus quadrato-ethmoidalis (see Pusey, 1938, fig. 8, *pqe*, and Text-fig. 7 E of this paper) laterally to the internal nostril and not postero-medially as Sæve-Söderbergh's theory requires.

(4) The pterygoid bone rudiment lies yet farther forwards in modern-type frog larvae and not behind the processus quadrato-ethmoidalis.

None of this evidence, however, is finally conclusive, but there are two further points.

(5) I am not satisfied that the interpretation of the impressions in the palatine bone, on which the theory of Sæve-Söderbergh and Watson turns, requires the presence of a commissure as well as of an antorbital process, with its anterior and posterior maxillary processes. It seems to me to be possible that the groove which Watson attributes to the presence of a commissure in *Branchiosaurus* (see his fig. 23) may have been due solely to the presence of the thickened posterior edge of the antorbital process, whilst the groove attributed to a posterior maxillary process may, in fact, have been due to the anterior maxillary process. In such a case the commissure would have been entirely absent. The slight distortion which these antorbital cartilages had undergone, in terms of the *Ranid* (adult) condition, would be due to the 'great forward extension of the sub-temporal fossa' in *Branchiosaurus*, which Watson himself mentions on p. 226. The same argument could apply to

Säve-Söderbergh's findings in the case of *Lyrocephalus*. Thus the whole system, which he shows as the antorbital process with its anterior and posterior processes in his figs. 7 and 8, etc., could well be the forwardly-arched roof of the nasal capsule standing out from the thickened edge of the actual antorbital process which he marks as the commissure. This roofing cartilage is apparently pierced by a hole due to the presence of a dorsal process of the dermopalatine bone, and it is this hole which gives rise to the idea of a separate antorbital process in front of it. Now there is a similar hole in the cartilaginous nasal roofing of *Discoglossus* (post-metamorphic stage) through which the lateral branch of the profundus nerve passes from the orbit into the capsule. If this hole were larger, *Discoglossus* would then parallel *Lyrocephalus* in this connexion, without, of course, retaining a commissure in this region.

It may be noted that neither Säve-Söderbergh nor Watson makes use of the evidence which Edgeworth (1925) has collected of an anterior connexion of the tip of the pterygoid process to the base of the antorbital process in the Urodeles. This comparison is, of course, ruled out for Säve-Söderbergh owing to his ideas of the separate origin of the Urodeles from the Fish. Edgeworth himself considered this Urodele connexion to be the homologue of the anuran commissure, but this homology is subject also to the very criticisms set out above and was, in fact, so criticized in my previous paper.

(6) Finally, to stress the primitively posterior origin of the commissure, one last point may be discussed, which seems to be the most important in this connexion. Gaupp (1893 and 1906) and Pusey (1938) have both drawn attention to the fact that at metamorphosis, in the modern-type frog larvae, a great part of the cartilage of the trabecula is destroyed by erosion. This erosion extends from under the ascending process behind, to the front border of the commissure in front. It certainly results in the breakdown of the attachment of the commissure to the skull and thus allows the whole quadrate bar to move freely backwards (Gaupp and Pusey). But it has always been a puzzle as to why so extensive a length of cartilage is destroyed, for not only is the outer part stripped off the whole central length

of the trabecula, but the entire bar is destroyed in places, so that the optic foramen becomes vertically confluent with the cranio-palatine foramen, and the oculomotor foramen with the primary carotid foramen. Now if fig. 17, Pl. 12, fig. 19, Pl. 13, and Text-fig. 7 D and E are studied, it will be seen that the commissure of *Ascaphus* is attached to the trabecular bar along just this very region which is destroyed in the modern-type frogs. De Villier's work (1934) shows that the commissure must be wholly detached from the trabecula at metamorphosis in *Ascaphus*, and this detachment is presumably achieved, as in *Rana*, by the erosion of the cartilages concerned. It would appear, therefore, that once this band of trabecular cartilage had become sensitive in the ancestor to respond to the stimulus which brings about the erosion, this sensitivity, together with the activating stimulus, has been retained by the descendants, so that the destruction has continued, though the need for it posteriorly has been superseded by the forward migration of the commissure. The phenomenon is thus an evolutionary, physiological relic which is important in that it supports the view that the commissure was primitively farther back in the head, as it is to-day in *Ascaphus*, and was not always an ethmoidal structure, as it is in modern-type tadpoles.

It is probable that a conclusive answer can finally be given to this question by a study of the larval stages of the other Liopelmid frog, *Liopelma*. If this frog, with its own peculiar terrestrial development, is like *Ascaphus* in possessing a commissure lying far back in the temporal region of the head, it would leave little doubt that this, and not the ethmoidal region, is the primitive site of this controversial structure.

(g) Changes in the Auditory Regions of Anuran Skulls.

Watson's researches (1940) make it clear that, whilst the commissure was moving forwards in phylogeny, the basitrabecular process was moving backwards, so that it finally came to underlie the front part of the auditory capsule, with which it became fused. This absorption of the process into the capsule

has had far-reaching effects on the anatomy of this part of the anuran skull. It probably led (1) to the suppression of the root of the process in *Liopelma* and all modern-type frogs, but not yet in *Ascaphus* (Text-fig. 7 D and E); (2) to the total suppression of the true cartilage floor of the capsule in *Ascaphus*. Then as the basitrabecular process became more and more reduced in development—only its outer end remaining as the pseudobasal process—(3) the prefacial commissure, present in the *Liopelmidæ* and the *Discoglossidæ*, was lost in all other modern-type frogs; and (4) the auditory capsule regained a true floor and side wall of its own above and beside the now conjoined facial and trigeminal ganglia, thus refilling the gaps made by the absence of the basitrabecular root and the prefacial commissure. This capsular floor is already appearing late in the larval development of the *Discoglossid* frogs; in the more modern-type frogs (e.g. *Rana*) it makes its appearance much earlier in ontogeny.

In the early phylogenetic stages of the incorporation of the basitrabecular process into the auditory capsule, the posterior corner of the palatoquadrate basal process must have kept contact with the outer end of the process (Text-fig. 7 B, C, and D), as it does to-day in *Ascaphus* with its complete basitrabecular process fused to a 'posterior basal process'. In *Discoglossus* a small remnant of this posterior basal process is still present (Text-fig. 6, *bpr*), but it is without a basitrabecular partner during larval life. At metamorphosis, however, it articulates, for a day or two only, with the young cartilage of the developing pseudobasal process (Text-fig. 7 E) (= the outer end of the basitrabecular process, Pusey, 1938) and then is later totally destroyed by erosion. This posterior basal process fails to develop in any other modern-type frog so far described.

Attention should be drawn to the extreme similarity, even to details, of the anterior part of the auditory capsule and the basal-basitrabecular articulation in *Ascaphus* and in the *Urodeles*. De Villiers (1934) has drawn attention to this similarity in the adult skull of *Ascaphus*. A comparison of fig. 10, Pl. 9, and figs. 11, 12, Pl. 10 with the sections of *Salamandra* figured by Goodrich (1930, fig. 453 c and d) brings

out the similarity; also fig. 17, Pl. 12, may be compared with Goodrich's fig. 261. Probably this common condition is arrived at by parallel evolution in the two orders. Such parallel evolution is much more likely to have gone on in stocks which were closely related genetically than in stocks independently derived from separate groups of Fish, in the manner suggested by the Swedish workers, Holmgren and Sæve-Söderbergh. There is this difference, however, that in the Urodeles the entire basal process must have passed backwards along with the basitrabecular process, without the anterior part having separated off to become a commissura quadrato-cranialis anterior. The articular region of the quadrate also remained far back in this order, so that the larva remained like the adult and no extensive metamorphosis of these parts was necessary.

(h) The Articular Region and the Body of the Quadrate.

In *Rana* and other modern-type frogs the articular region of the tadpole quadrate lies under the olfactory foramen; in *Ascaphus* it lies just behind the optic foramen. A comparison of fig. 8, Pl. 36, of Pusey, 1938, with fig. 15, Pl. 11, and fig. 19, Pl. 13, shows how the change has been brought about. From the evidence of Craniates as a whole we know that primitively the body of the quadrate was a mass of cartilage lying under the auditory capsule. In *Ascaphus* this block has been elongated anteriorly so that the quadrate is a fairly long band when seen from above and its articular region has moved in phylogeny along the under edge of the pterygoid process (Text-fig. 7 d). In the change from the *Ascaphus* to the *Ranid* condition, the quadrate bar, as a whole, has moved forwards, carrying the commissure and the pterygoid process with it, so that the front limits of these two structures have advanced from a position midway between the optic and olfactory foramina, in *Ascaphus*, to a position under the olfactory foramen in *Rana*; Text-fig. 7 e shows a midway stage in this evolution. But further, the articular region has moved forwards much more rapidly than the main body of the quadrate, passing from the level of the optic foramen to a position in front of

the olfactory foramen (Text-fig. 7 D and E). It has done this by developing, in successive ontogenies, further and further forwards along the under side of the pterygoid bar, obscuring, as it did so, all but the quadrato-ethmoidal tip of this bar in the substance of the commissure and the body of the quadrate. During this general advance the cartilage of both the quadrate bar and the commissure, as represented in *Ascaphus*, has been lightened posteriorly by the formation in them of the subocular vacuity. As suggested in the previous paper, the subocular shelf (? of the trabecula) and Gaupp's processus pseudo-ptyergoideus probably represent remnants of this suppressed cartilage in the modern-type tadpoles.

(i) The Otic Processes.

Whilst the main body of the quadrate was moving forwards in phylogeny, its outer, upper border—the otic process—was similarly being elongated in a forward direction; all the time, however, it retained its connexion with the wall of the auditory capsule behind in the form of the 'tadpole otic process'. In *Ascaphus* the front border of the ancestral otic process has reached forwards to the level of the oculomotor foramen, whereas in *Rana* it has reached to the olfactory foramen (where it is represented by the front border of the quadrate muscular process; see Pusey, 1938, p. 523). Thus in all frogs the whole lateral border of the long quadrate bar represents the much narrower otic process of the ancestor and, as usual, *Ascaphus* has remained at the half-way condition.

An examination of the figures of *Ascaphus* shows that there are two regions in which the posterior part of the quadrate bar is fused to the wall of the auditory capsule. These regions are parted by a foramen (*forcl*) through which a vein passes downwards to join the vena capitis lateralis, after draining blood from the overlying adductor jaw muscles. An examination of *Rana temporaria* tadpoles in sections shows that a vein of precisely similar relations runs backwards from the more forwardly-lying jaw muscles, to pass downwards over the hind edge of the quadrate bar, in front of the tadpole otic process, to join the

lateral head vein. For this reason I have marked the posterior fusion in *Ascaphus* as the tadpole otic process. The anterior fusion seems to be an additional strengthening of the autostyly of the jaws achieved by a fusion of the tip of the auditory capsule to the hinder border of the ascending process. Unfortunately this critical vein seems to be wholly absent in *Discoglossus* and in *Bombina*, where, too, the tadpole otic fusion is also absent. In *Discoglossus*, however, the back of the quadrate bar is cupped and fits very closely against the rounded end and side of the auditory capsule, as a ball and socket joint. There is, in fact, no connexion between the two, although this could easily be achieved and the condition points to the way in which this fusion has been brought about in the admittedly specialized *Ascaphus*. The evidence could, of course, be read in the reverse way: the additional fusion could be thought of as the primitive condition in the ancestral frog larva and as only recently lost in *Discoglossus*; but it seems more probable that it is one of the secondary features in *Ascaphus*.

(j) The Muscular Process.

The elongated otic process of *Ascaphus* gives origin to certain mandibular and hyoid muscles. The two hyoid muscles mainly concerned are the suspensorio-angularis muscle (*sam*) to the jaw and the orbitohyoideus muscle (*ohm*) to the ceratohyal. They take origin all along the under side and outer edge of the palatoquadrate bar from the roof of the quadrate tunnel in front to the tadpole otic process behind. There is thus no specialized and sharply upraised muscular process which, in modern-type frogs, is such a characteristic of the larval palatoquadrate. None the less, the front part of the quadrate bar over the tunnel is slightly raised above the general level, and I have consequently marked it as a muscular process in the figures, although it is but an incipient one.

The fact that the muscular origins are widespread and not localized in *Ascaphus* upholds the view of an elongated otic process in this genus. We now, however, must face the question of why there is a well developed and localized muscular process

in the modern-type tadpoles. The answer turns on three points. (1) The ceratohyal has moved to a more forward position in the modern-type frogs; (2) it has shortened and its posterior corner has been brought forwards relative to the bar as a whole; (3) as a result the primitive origins and insertions, particularly of the orbitohyoideus muscle, would have been brought much closer together unless a muscular process was developed to carry the origin up and away from the insertion. It is a well known fact that a vertebrate muscle fibre can only reduce its length by contraction to a certain fixed fraction of its resting length. Consequently, if a moving part of the skeleton is to be activated to the same extent in two animals with different anatomical relations of the skeletal parts, the length of the muscle fibres concerned must be maintained above a certain minimum value, and this can best be achieved by keeping the origin and insertion at a suitable distance apart. A comparison of fig. 19, Pl. 18, of this paper with fig. 1, Pl. 33, of my previous paper makes the position clear. In *Ascaphus* the fibres of the orbitohyoideus muscle run a sufficiently long course to the tip of the ceratohyal to allow of the necessary contraction without the presence of a tall muscular process. If, however, in *Rana* and the modern-type frogs there was no upraised muscular process the muscle fibres would be very much shortened and would therefore be less effective. Consequently, a muscular process is developed in the modern-type frogs to keep the origins and insertions of the muscles suitably parted from one another.

The change in orientation of the ceratohyal in advanced frogs is obviously a part of the general tendency to forward migration of all splanchnic structures. The reduction in length of the bar obviously prevents its posterior and now outer end from projecting far out beyond the overlying quadrate in a way that would upset the streamlining of the larval head. When the ceratohyal thus came to underlie one part only of the quadrate bar in the modern-type frogs, instead of lying beneath the greater part of the bar as in *Ascaphus*, the origins of the orbitohyoideus and other muscles also came to be localized above it around the edges of the muscular process, instead of

being spread out all along the quadrate otic process. Thus, there can be no doubt that the presence of a muscular process in the modern-type frog tadpole is but a necessary result of the general forward movement of the structures underlying the skull. The actual muscular process is made by a modification of the front border of the elongated ancestral otic process, as was suggested in a previous paper (Pusey, 1938, p. 523).

(k) The Origins of the Adductor Jaw
Muscles.

The same argument, as set out above, can be applied to the length of the muscles fibres and to the variations in the origins of the mandibular muscles of *Ascaphus* on the one hand and of the advanced frogs on the other. The larval *Ascaphus* is unique among frogs in that the superficial and deep posterior levator muscles of the jaw take origin far back on the roof of the auditory capsule, as well as on the quadrate bar. In all other frog tadpoles these muscles arise only from the hinder edge of the quadrate bar and its ascending process. Correlated with this difference is the different position of the lower jaw in the two opposing types of organization. In *Ascaphus* the lower jaw lies below and just in front of the level of the optic foramen (see fig. 19, Pl. 13), whereas in other frogs it lies under the region of the olfactory foramen (see fig. 1, Pl. 33, Pusey, 1938). The distance between these two positions (as measured on either of the types concerned) is approximately equal to the distance between the sites of origin of the muscles in the two forms. Thus, since the jaw lies more posteriorly in *Ascaphus*, the muscle origins must also lie farther back in order to give the muscles a sufficient length to carry out effective contraction. But when, as in the modern-type frogs, the jaw moved forwards, carrying with it the muscular insertions, the muscular origins were able to descend from the capsular roof to a more forward position, without affecting the degree to which the muscles could shorten. Thus this difference in muscular origins is also

related to the forward migration of the splanchnic structures.

(l) The Ascending Process of the Palatoquadrate.

The ascending process is unusually broad and strong in *Ascaphus* and is attached to the pila antotica and the orbital cartilage high up along the side of the skull. This primitive high-level attachment is retained in the Discoglossidae; in other frogs, however, the attachment is rather to the trabecula, or the base of the pila. The process appears to be very short in *Ascaphus* because its hind border is completely fused to the front face of the auditory capsule almost as far laterally as the larval otic process. The normal long chink between the process and the capsule is only represented medially by the dorsal division of the prootic foramen (*ftd*) and laterally by the small venous foramen (*frcl*). The presence of this additional fusion bears out the view put forward in a previous paper (Pusey, 1938, p. 534) that the 'bony bridge' described by de Villiers (1934) in the adult skull of *Ascaphus* is but the root of the ascending process which is retained through the period of metamorphosis without the destruction which is the rule in modern-type frogs. It will be interesting to observe what bone ossifies this bridge after metamorphosis. Will it prove that *Ascaphus* adds to its other primitive characters the retention of an independent epipterygoid bone, or will it be found that the process is ossified from the prootic centre? Watson (1940) has found a separate epipterygoid bone in *Miobatrachus*, but this region of the skull of *Protobatrachus*, with its foramina, is unfortunately not well known.

(m) The Larval Otic Process of *Xenopus*.

Before finally leaving the above topic it may be well to make a suggestion about the otic attachments of the larval palatoquadrate of *Xenopus laevis* to the auditory capsule. Dr. Patterson (1939) gives excellent reconstructions in her recent paper on 'The Head of *Xenopus laevis*', and her figures show conditions at least superficially similar to those of *Ascaphus*. That is

to say, that in *Xenopus* there are two fusions of the back of the palatoquadrate bar to the auditory capsule, one to the anterior tip of the capsule and one more postero-laterally to its side wall, the two being parted by a foramen over the thymus gland; see her fig. 14, Pl. 12. Her fig. 23, Pl. 13, makes it clear that neither fusion is present in the early (10 mm.) larva, but that each is already well formed in the 28 mm. stage (fig. 27 *a* to *c*, Pl. 15) and is further consolidated in the 60 mm. stage (fig. 24, Pl. 14). She marks the antero-medial of these connexions as the larval otic process and in this she follows the lead of Kotthaus (1933), Edgeworth (1935), and de Beer (1937). It seems to me more probable, however, that this title should be reserved for the postero-lateral connexion, for this latter seems to be made with a more usual part of the capsular wall and bears more typical relations to the thymus gland, the lateral head vein, the branches of the IX nerve, and the ramus connecting it to the VII nerve; there is also now the evidence from *Ascaphus*. Unfortunately, the vein which I have used as the critical test for the otic process in *Ascaphus* and *Rana* is apparently absent in *Xenopus*, judging from Miss Patterson's careful drawings of selected sections (figs. 6 to 17, Pls. 11 and 12). The adductor jaw muscles, which this vein drains in other frogs, are poorly developed and have very anteriorly placed origins which fail to reach back to anywhere near the capsule in this genus (cf. her figs. 8 and 9). I raised this point in a letter to Miss Patterson and, in reply, she was kind enough to make and send to me a wax-plate reconstruction of the auditory region of her 60 mm. stage, for which I would again thank her here. She still, however, maintained the view published in her paper; yet a study of her model leaves me convinced that it is none the less the postero-lateral connexion which is the larval otic process in *Xenopus*.

I would also like to correct a statement which Miss Patterson makes on p. 179. What she there claims to be the 'quadrato-ethmoidal cartilage' is surely a chondrified trabecular-quadrato ligament and not a chondrified quadrato-ethmoidal ligament (see particularly her figs. 6, 23, 24, and 27 *a*). A comparison of her fig. 27 *c* with my fig. 19, Pl. 13, taken in conjunction with

remarks made about this ligament in other primitive frogs, on p. 112 of this paper, suggests, as at least a possibility, that the tentacular cartilage of *Xenopus* is the homologue of the lateral cartilage wing (*clsl*) of the supra-rostral system of *Ascaphus*.

(n) The Roof of the 'Quadrate Tunnel'.

Before leaving the palatoquadrate I would draw attention to the exact similarity of anatomical relations between the cartilage forming the roof of the quadrate tunnel (*at*) in *Ascaphus* (see p. 121) and the small, sickle-shaped cartilage in, say, *Rana* or *Discoglossus*, which is the first rudimentary trace of the annulus tympanicus. It is worth remembering that Gaupp (1893 and 1906) describes the formation, in *Rana*, of a mesenchyme cloud from the base of the muscular process at its front edge, in early stages. This cloud remains throughout the larval life surrounding a branch of the carotid artery and touching the quadrate above and below it. It becomes denser as metamorphosis approaches and the annular cartilage condenses only from its outer part, laterally to the artery. If, however, the whole cloud were to chondrify, it would complete a tunnel as in *Ascaphus*. Now *Ascaphus* has no middle ear apparatus nor tympanic ring in the adult (de Villiers, 1934), a condition which must certainly be one of secondary loss, at least as far as the columella system is concerned, so that it would be unwise to draw any far-reaching conclusions from this otherwise primitive frog as to the ancestral origin of the annulus, but the condition may perhaps be taken as further evidence to support Gaupp's view that the annulus, which is peculiar to the Anura, is a separated part of the quadrate bar.

(o) The Supra-rostral System.

Without further embryological evidence from earlier stages little can be said of the homologies of the parts of this system to the structures in other Vertebrates than the Anura. I have sought to compare the structures with parts of the nasal apparatus of Urodeles and other types, but have failed. There

need be no surprise that the system is divided into one median and two lateral parts, for this condition is foreshadowed by the evidence collected together from other frogs by van Seters (1922) and shown in his fig. 10. I have found that in *Discoglossus* the system is also deeply cleft into three sections which are, however, fused to one another (Text-fig. 7 B). It appears, therefore, that the supra-rostral system of the ancestor was divided up into three pieces (or if the medial piece is really of dual origin, possibly into four pieces). It is rather (1) the extreme posterior position of the whole system and (2) the rigid attachment of its median piece to the trabeculae, which are of interest in *Ascaphus*. The posterior position is to be expected to some extent from what has been said on pp. 146 to 148, but in *Ascaphus* this posterior position seems almost to be exaggerated in the way expected if the criticism given on p. 148 et seq. were valid. That is to say, that the whole apparatus seems to be further back than it would be if it were just a slightly modified arrangement of the tips of the trabeculae. Further, the fusion of the medial piece to the under sides of the trabeculae seems also to be very much exaggerated and to be carried also somewhat far back. These backward displacements are probably secondary characters related to the peculiar behaviour of the tadpole. They are part of a general tendency, discernible also in other structures, to supply the mouth and pharynx cavity with the maximum skeletal support on all sides and particularly underneath, so that the sucker has a rigid roof which can be lifted up as a whole and can withstand the atmospheric pressure. If fig. 15, Pl. 11 is superimposed on fig. 21, Pl. 14, so that the jaws in the two coincide, it will be seen that the mouth and pharynx cavities are largely ringed round with cartilage struts which leave few membranous spaces between them. (1) The posterior jaw cartilages are broad plates; (2) the space between the anterior jaw cartilages and the ceratohyals is supported by the unique posterior spurs of the jaw cartilages; (3) the ceratohyals are broad plates and the medial part of the hyobranchial apparatus is also much consolidated; and (4) the gill bars, particularly the anterior three pairs, are also widened antero-posteriorly. Thus the peculiar box-like structure of the supra-

rostral system, in its medial part, supplies the anterior section of this general basket work, which as a whole is presumably a secondary character related to the sucker mechanism.

8. THE CHARACTERS WHICH ASCAPHUS SHARES WITH THE
URODELES.

(1) Short trabecular horns.

(2) The nasal sacs lie laterally to the olfactory foramina and not largely in front of them.

(3) The anatomy of the auditory capsule at its anterior end, e.g. a complete basitrabecular process acts as the effective anterior floor and is intimately fused to the capsular walls; the true capsular floor is present only as membrane; a joint anterior acoustic foramen and exit for the facial nerve which appears to run through the capsular cavity to separate foramina for its hyomandibular and palatine branches.

(4) A prefacial commissure is present.

(5) Absence of a taenia tecti medialis and a t. t. transversalis in both larva and adult.

(6) Parachordal cartilage underlies the notochord (as well as overlying it in the usual anuran manner, so that the notochord is encased on all sides in *Ascaphus*).

(7) The relatively posterior position of the palatoquadrate and its parts, and of many other splanchnic structures (see p. 146 et seq.).

(8) The pterygoid process is unobscured below in the body of the quadrate.

(9) The posterior position and the extent of the pterygoid bone-rudiment (= ligamentum quadrato-ethmoidale).

(10) The high-level attachment of the ascending process of the quadrate to the pila antotica and to the orbital cartilage and the retention of its root throughout adult life.

(11) The articulation between the quadrate basal process (in *Ascaphus*, only its posterior part) and a complete basitrabecular process of the cranial floor.

(12) The absence of a well developed muscular process from the otic process of the quadrate.

(13) The posterior position of the whole hyobranchial

apparatus and the more antero-posterior, rather than medio-lateral, orientation of the gill bars.

(14) The absence of a processus branchialis between the bases of the IInd and IIIrd gill bars, and the freedom of the IVth arch from the hypobranchial plate.

(15) The presence of forked 'urobranchial' prongs on the under side of the basibranchial copula (copula II).

(16) The mandibular muscles are not greatly subdivided; thus there are five pairs only (*Ascaphus*) instead of ten pairs (modern-type frogs); there are five pairs in *Urodeles*.

(17) The intermandibularis muscle partly underlies the inter-hyoideus muscle.

(18) Levator mandibulae anterior (pterygoid) fibres, though fused with L. m. posterior profundus fibres, arise high up on the orbital cartilage in front of the quadrate ascending process and the exit of nerves V 2 and 3 (and not from the front face of the auditory capsule below the ascending process as in modern-type frogs).

(19) The hyoid muscles are not greatly subdivided; thus there are five pairs in *Ascaphus* instead of seven pairs as in the modern-type frogs; the *Urodeles* have from four to six pairs.

(20) Of the five pairs of hyoid muscles mentioned above, one pair—the branchio-hyoideus externus—is shared with the *Urodeles*, but is absent from all modern-type frogs except the *Discoglossidae*.

(21) The posterior origins of the Levatores arcuum branchialium muscles on the posterior wall of the auditory capsule (I and II, and III in part) and on the sheaths of the neck muscles (II and III in part, and IV).

(22) Subarcualis rectus IV passes from arch IV to arch I (but not to a branchial process as in other frogs).

(23) *Ascaphus* is more primitive than the *Urodeles* in possessing Subarcuales obliqui muscles arising on arches V and IV, and is like them in having similar muscles arising on arches III and II. Modern-type frogs have only one of these muscles arising from arch II (transversus ventralis II, of Edgeworth). *Ascaphus* is like the *Urodeles* in that these muscles are inserted on the forked urobranchial prongs of the basibranchial copula.

(24) The persistence of a *Transversus ventralis* IV muscle in old larvae of *Ascaphus*. This muscle develops, but is soon lost, in the ontogeny of modern-type frogs.

(25) See p. 145 for a ? abnormality of the *geniohyoideus* muscle in this specimen of *Ascaphus*.

(26) The attachment of the *Rectus cervicis* muscle to the urobranchial prongs of the basibranchial copula.

9. THE CHARACTERS WHICH ASCAPHUS SHARES WITH DISCOGLOSSUS.

(1) The supra-rostral system is cleft into three parts, but these are not separate from one another in *Discoglossus* as they are in *Ascaphus*.

(2) The attachment of the trabecular-quadrate ligament to the lateral wing of the supra-rostral and not to the tip of the trabecular horn.

(3) Certain auditory capsular arrangements, e.g. the common entrance to the anterior acoustic foramen and the facial nerve tunnel; the facial nerve is free in the apparent capsular cavity in young larvae of *Discoglossus*; separate foramina for VII palatine and hyomandibular.

(4) A prefacial commissure is present.

(5) Absence of a *taenia tecti medialis* in the cartilage skull roof.

(6) The high-level attachment of the quadrate ascending process to the *pila antotica* and to the orbital cartilage.

(7) The close association of the anterior tip of the auditory capsule with the posterior border of the ascending process and the body of the quadrate as a ball and socket joint; compare the actual fusion in *Ascaphus*.

(8) The presence of a small 'posterior basal process' on the quadrate (see Text-fig. 6, p. 155).

(9) The absence of a *processus branchialis* joining the IIrd and IIIrd gill bars. There is, however, an incipient process projecting forwards from the IIIrd bar in *Discoglossus*.

(10) A dorso-ventrally deep, but laterally narrow urobranchial keel on the basibranchial copula of *Discoglossus*, not, however forked at its posterior end as in *Ascaphus*.

(11) Presence of a *branchio-hyoideus externus* muscle.

(12) Absence of a hyoangularis muscle.

(13) Posterior origins of the suspensorio-angularis and suspensorio-hyoideus muscles.

(14) The quadrato-angularis muscle arises medially to the ceratohyal-quadrato articulation, not laterally to it.

(15) The Subarcualis IV muscle passes from arch IV to arch I and not to a processus branchialis.

(16) The simple form of the Subarcualis I muscle.

10. SUMMARY.

This paper gives the first account of the larval cranial anatomy of either of the genera of Liopelmid frogs.

A single, partly grown larva of *Ascaphus truei*, Stejneger, has been studied in transverse sections and in two-dimensional reconstructions. Its chondrocranium, jaws, gill arches, and head muscles are described and figured. Comparisons are made throughout with similar structures of Urodeles and certain other frogs, particularly *Discoglossus pictus* and *Rana temporaria*. A summary of the characters which *Ascaphus* shares with the Urodeles is given on pp. 175-7 and with *Discoglossus* on pp. 177-8. The reader is referred to these lists as an important part of this summary.

Noble (1931, &c.) considers *Ascaphus* (with *Liopelma*) to be one of the two most primitive living frogs. The findings of this paper are in full agreement with this view. Thus larval *Ascaphus* is shown to be a persistently primitive 'link-animal' whose cranial structures, in almost every case, differ from those of other frogs—often radically—and throw much light on the evolution of the modern-type frog tadpole from the unknown (larval) ancestor.

Ascaphus is shown to have more characters in common with the Urodeles than any other frog larva yet described. Most of these are probably a simple retention of an ancestral Amphibian plan which led on to the frogs and Urodeles (contrast the writings of Holmgren and Sæve-Söderbergh). Others seem to link these two orders even more closely together. Such are: (1) The presence of 'urobranchial' prongs on the basi-branchial copula and the attachment to them of Subarcuales

obliqui and Recti cervicis muscles; (2) the presence of a pair of Branchio-hyoideus externus muscles and other similarities of the musculature. The relationship of *Ascaphus* to the *Gymnophiona* is far less marked.

Ascaphus, however, has remained more primitive than the present-day *Urodeles* by retaining: (1) a ? Vth gill bar, with its Subarcualis rectus and S. obliquus muscles, and (2) four pairs of S. obliqui muscles instead of two. In these points it is, in fact, the most primitive living tetrapod.

Ascaphus is, however, somewhat specialized in relation to a sucker mechanism and to a peculiar method of larval progression which it employs. These have led to an exaggerated autostyly of the palatoquadrate which has developed an additional fusion to the anterior tip of the auditory capsule; to a rigid fusion of the central part of the supra-rostral system to the skull; to the general heavy build of the head cartilages and to the great size of several of the mandibular and hyoid muscles; to a general consolidation and widening of parts of the hyobranchial apparatus; to a widening of the posterior jaw cartilages and the development from them of unique posterior spurs. A pre-oral mouth cavity and a long 'posterior narial tube' to the inner nostril are also parts of this specialization.

Among the frogs *Ascaphus* is shown to be most nearly related to the *Discoglossidae*, which appear to have been derived from an ancestor with many *Ascaphus*-like characters. This is in further agreement with Noble's classification and is particularly true of *Discoglossus pictus*.

Ascaphus is unique among frogs in the posterior position of its splanchnic head structures; see the list on p. 147.

A forecast is made of the probable evolution of the modern-type tadpole's jaw system from that of the unknown ancestor and this is diagrammatically summed up in Text-fig. 7, pp. 158-9.

Evidence is collected to show that the 'anterior basal process' (= commissura quadrato-cranialis anterior) is not an ethmoidal structure by origin, as has been held up to now, and consequently Sæve-Söderbergh's use of it to explain an ethmoidal structure in a *Stegocephalian* Amphibian is criticized.

An account of the muscles has been given in summary form

on pp. 126 to 146 and cannot be further condensed here. But it may be noted that Edgeworth's theories (1935) of the primitive muscular content of a single branchial segment break down when applied to *Ascaphus*. It is now probable that a single segment could simultaneously contain a *Subarcualis rectus*, a *S. obliquus*, and a *Transversus ventralis* muscle. Further, Edgeworth's term '*Transversus ventralis II*' must be changed to '*S. obliquus II*' in the frogs and his '*S. rectus IV*' must probably be changed to '*S. recti IV, III, and II*' in the Urodeles.

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12. ABBREVIATIONS USED IN THE FIGURES.

a, artery; *ac*, auditory capsule; *ajc*, anterior jaw cartilage (= cartilago labialis inferior, Gaupp); *ap*, anterior pit in cranial floor; *asc*, anterior semicircular canal; *at*, arterial tunnel through quadrate; *b*, brain; *ba I-IV*, branchial arches I-IV; ? *ba V*, probable Vth branchial arch (= spiculum IV); *bbc*, basibranchial copula; *bhc*, basihyal copula; *bhem*, branchio-hyoideus externus muscle; *bpr*, basal process of quadrate (posterior part); *bt*, basitrabecular process; *bv*, blood-vessel; *c*, carotid artery; *cb*, cartilage bridge; *cbm I to III*, Ist to IIIrd constrictores branchiales muscles; *cbr*, branch of carotid artery; *cf*, cranial floor; *ch*, ceratohyal; *cl*, cartilage ledge below nasal sac; *clsl*, lateral wing of supra-rostral cartilage (part of cartilago labialis superior, Gaupp); *clsm*, median part of supra-rostral cartilage (part of cartilago labialis superior, Gaupp); *con*, conus; *cq*, cranio-quadrate passage; *cqa*, commissura quadrato-cranialis anterior (anterior part of quadrate

basal process); *ct*, trabecular horn; *dbrn*, diaphragmato-branchialis muscle; *de*, deep epidermis; *dm*, dense mesenchyme; *dpba I*, dorsal process of Ist branchial arch; *eb*, epithelial band; *en*, external nostril; *erm*, external rectus muscle; *f*, region of fusion of quadrate with auditory capsule; *faa*, foramen acusticum anterius; *fam*, median acoustic foramina; *fap*, foramen acusticum posterius; *fc*, foramen cranio-palatinum; *fcp*, foramen caroticum primarium; *fen*, foramen endolymphaticum; *ff*, facial foramen (as yet undivided); *fhm*, foramen for hyomandibular branch of facial nerve; *fj*, foramen jugulare; *fo*, fenestra ovalis; *foen*, foramen oculomotorii; *fol*, foramen olfactorium; *fon*, foramen opticum; *fpi*, foramen perilymphaticum inferius; *fps*, foramen perilymphaticum superius; *ft*, trigeminal foramen; *ftd*, trigeminal foramen (dorsal division for V_2 and V_3); *ftv*, trigeminal foramen (ventral division for V_1); *fu*, fusion of processus posterior hyalis with Ist branchial arch; *fucl*, foramen for branch of vena capitis lateralis; *g*, glottis; *ghm*, geniohyoideus muscle; *hbp*, hypobranchial plate; *hbp*, horny tooth-plate of upper 'lip'; *ifrm*, inferior rectus muscle; *ilm*, interhyoideus muscle; *in*, internal nostril; *iom*, inferior oblique muscle; *ipm*, intermandibularis posterior muscle; *irm*, internal rectus muscle; *l*, ligament; *la*, left auricle; *labm I and II*, Ist and IInd levatores arcuum branchialium muscles; *labm IV*, levator (+constrictor) IV muscle; *lac*, 'cartilage ledge of auditory capsule', de Villiers (= outer edge of basitrabecular process and post-palatine commissure); *lc*, lateral semicircular canal; *ls*, lower (posterior) 'lip', forming sucker; *lmam*, levator mandibulae anterior articularis muscle; *lmpm*, levator mandibulae posterior profundus muscle, ? + levator mandibulae anterior muscle; *lmsm*, levator mandibulae posterior superficialis muscle; *lq*, quadrato-ethmoidal ligament (= rudiment of pterygoid bone); *ls*, lymph space; *lst*, tendon of levator mandibulae posterior profundus muscle; *lt*, tendon of levator mandibulae posterior superficialis muscle; *mfc*, membranous true floor of auditory capsule; *mo*, mouth opening; *msr*, membranous skull roof; *mtr*, membranous temporal roof; *n*, notochord; *nc*, notch in quadrate for articulation of ceratohyal; *nm*, musculature of neck; *nnt*, notch for 'posterior narial tube'; *ns*, nasal sac; *nsr*, V-shaped notch in supra-rostral cartilage; *oa*, occipital arch; *oac*, outline of auditory capsule; *oc*, orbital cartilage; *ocr*, occipital condyle; *oec*, open end of cranium; *ohm*, orbito-hyoideus muscle; *op*, operculum; *pa*, ascending process of quadrate; *paq*, articular region of quadrate; *pbc*, palatine branch of carotid artery; *pcqa*, posterior border of commissura quadrato-cranialis anterior; *pf*, foramen for palatine branch of facial nerve; *pfc*, prefacial commissure; *php*, pouch of pharynx; *pia*, pila antotica; *pit*, pituitary gland; *pitf*, pituitary fossa; *pjc*, posterior jaw cartilage (cartilago Meckelii, Gaupp); *pm*, preoral buccal cavity; *pmq*, muscular process of quadrate; *pnt*, 'posterior narial tube'; *pot*, processus oticus quadrati (larval); *ppc*, post-palatine commissure; *pqe*, processus quadrato-ethmoidalis; *pre*, pars reuniens; *psp*, pseudo-basal process (= detached outer end of basitrabecular process); *psq*, posterior spur of quadrate; *pt*, profundus tunnel; *ptc*, pterygoid process of quadrate; *q*,

quadrate; *qdm*, quadrato-angularis muscle; *ra*, right auricle; *ram*, rectus abdominis muscle; *rcm*, rectus cervicis muscle; *rcsm*, rectus cervicis superficialis muscle; *s*, postero-ventral spur of posterior jaw cartilage; *sam*, suspensorio-angularis muscle; *saq*, surface of ceratohyal articulating with quadrate; *saom II to V*, IInd to Vth subarcuales obliqui muscles; *sarm I, IV, and V*, Ist, IVth, and Vth subarcuales recti muscles; *shm*, suspensorio-hyoideus muscle fibres; *soc*, subopercular cavity; *som*, superior oblique muscle; *sov*, subocular vacuity; *sp II and III*, spicula over IInd and IIIrd branchial arches; *srn*, superior rectus muscle; *t*, trabecula cranii; *tac*, tip of auditory capsule; *tc I-II, and III-IV*, terminal commissures between branchial arches I and II and III and IV; *tg*, thymus gland; *thg*, thyroid gland; *tl*, horny teeth of lower 'lip'; *tm*, taenia tecti marginalis; *tgl*, trabecular-quadrate ligament; *ts*, tectum synoticum (incomplete); *tul*, horny teeth of upper 'lip'; *tvm IV*, transversus ventralis IV muscle; *ul*, upper (anterior) 'lip'; *uls*, upper anterior 'lip', forming sucker; *upbc*, urobranchial prong of basibranchial copula; *v*, ventricle; *va*, ventral aorta; *vl*, vena capitis lateralis; *vlb*, branch of vena capitis lateralis; *vn*, valve of external nostril.

I, olfactory nerve; *I b*, branch of olfactory nerve; *II*, optic nerve; *III*, oculomotor nerve; *IV*, abducens nerve; *V₁*, profundus branch of trigeminal nerve; *Vg*, trigeminal ganglion; *Vr*, root of trigeminal nerve; *V₁ b*, branch of profundus nerve; *V₂ b*, branch of maxillary branch of trigeminal nerve; *V₃ b*, branch of mandibular branch of trigeminal nerve; *VII b*, branch of facial nerve; *VII g*, facial ganglion; *VII hm*, hyomandibular branch of facial nerve; *VII p*, palatine branch of facial nerve; *VII r*, root of facial nerve; *VII-VIII r*, joint root of facial and auditory nerves; *VIII b*, branch of auditory nerve; *VIII g*, auditory ganglion; *IX b*, branch of glossopharyngeal nerve; *IX rc*, ramus communicans from glossopharyngeal to facial nerve.

13. DESCRIPTION OF PLATES 6-14.

All figures are drawn or reconstructed from a series of transverse sections of a single larval specimen of *Ascaphus truei*: overall length 28 mm., tail length c. 18 mm., no hind legs present, having completed about one half to two-thirds of its larval life.

(The sections were mounted in rows of nine, with four rows to each slide.)

Relative to the sections shown in figs. 1, 2, 3, Pl. 6; figs. 4, 5, Pl. 7; figs. 6, 7, Pl. 8; figs. 8, 9, 10, Pl. 9; figs. 11, 12, 13, 14, Pl. 10, and in Text-figs. 1 to 5, all reconstructions are reversed, so that the animal's right side has become the apparent left.

PLATE 6.

Fig. 1.—Slide 4, row 1, section 2; through line AA of the reconstructions: fig. 15, Pl. 11, fig. 17, Pl. 12, and fig. 19, Pl. 13.

Fig. 2.—Section 4-3-4; through line BB of the reconstructions.

Fig. 3.—Section 5-3-2; through line CC of the reconstructions.

PLATE 7.

Fig. 4.—Section 6-1-7; through line DD of the reconstructions, including fig. 21, Pl. 14.

Fig. 5.—Section 6-4-7; through line EE of the reconstructions.

PLATE 8.

Fig. 6.—Section 7-3-6; through line FF of the reconstructions.

Fig. 7.—Section 8-3-1 (in part); through line GG of the reconstructions.

PLATE 9.

Fig. 8.—Section 9-1-1; through line HH of the reconstructions.

Fig. 9.—Section 9-3-6 (in part); through line II of the reconstructions.

Fig. 10.—Section 10-1-7 (in part); through line JJ of the reconstructions.

PLATE 10.

Fig. 11.—Section 10-2-2 (in part); through line KK of the reconstructions.

Fig. 12.—Section 10-3-1 (in part); through line LL of the reconstructions.

Fig. 13.—Section 11-1-5 (auditory region); through line MM of the reconstructions.

Fig. 14.—Section 11-2-4 (auditory region); through line NN of the reconstructions.

PLATE 11.

Fig. 15.—Reconstruction of the chondrocranium and jaws: seen dorsally and 23° from the left side. (Fig. 15, Pl. 11 may be fitted to fig. 21, Pl. 14.)

Fig. 16.—Reconstruction of the supra-rostral system and anterior end of the cranium: seen from in front. Fig. 16 may be fitted to Fig. 20, Pl. 13.

PLATE 12.

Fig. 17.—Reconstruction of the chondrocranium and upper jaw systems; the lower jaw has been removed: seen ventrally and 10° from the animal's left side.

Fig. 18.—As Fig. 16, but seen from behind.

PLATE 13.

Fig. 19.—Reconstruction of the chondrocranium, jaws, and ceratohyal; lateral view.

Fig. 20.—Reconstruction of the chondrocranium and lower jaw: seen from in front. The anterior end of the cranium and the supra-rostral system

PLATE 14.

(see figs. 16 and 18) have been cut away. Fig. 16, Pl. 11 may be fitted to Fig. 20.

Fig. 21.—Reconstruction of the lower jaw and hyobranchial cartilages: seen dorsally and 23° from the left side. (Fig. 21 may be fitted to fig. 15, Pl. 11.)

Fig. 22.—Reconstruction of the auditory capsule: seen from in front. The front of the cranium and the palatoquadrate have been cut away.

Fig. 23.—As fig. 22, Pl. 14, but with parts of the nerves V, VII, VIII, and IX added.

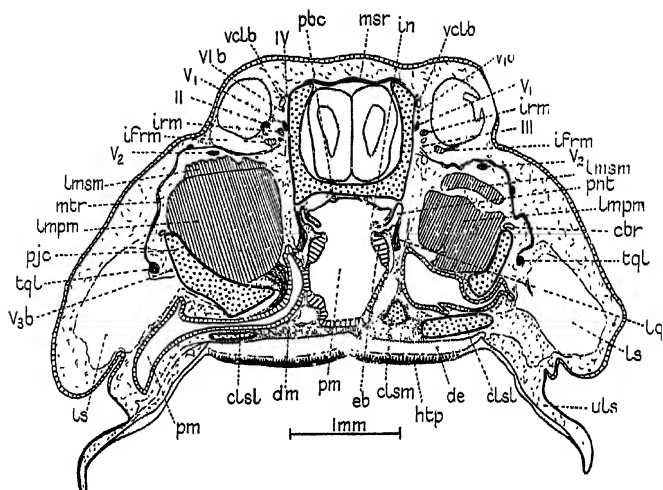


FIG. 4

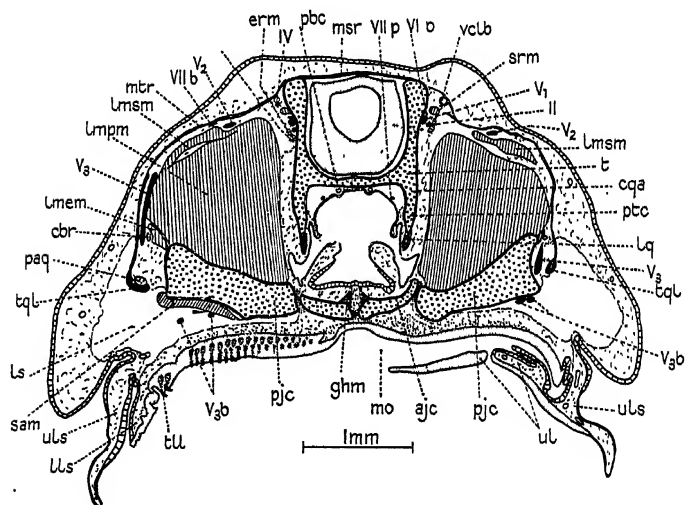


FIG. 5

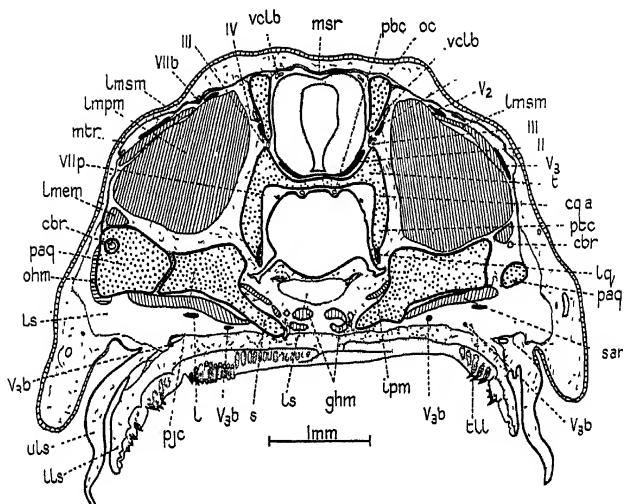


FIG. 6

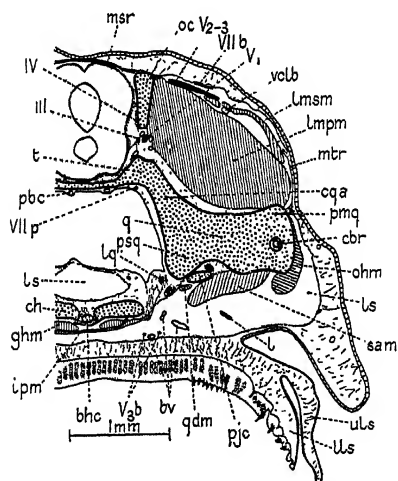


FIG. 7

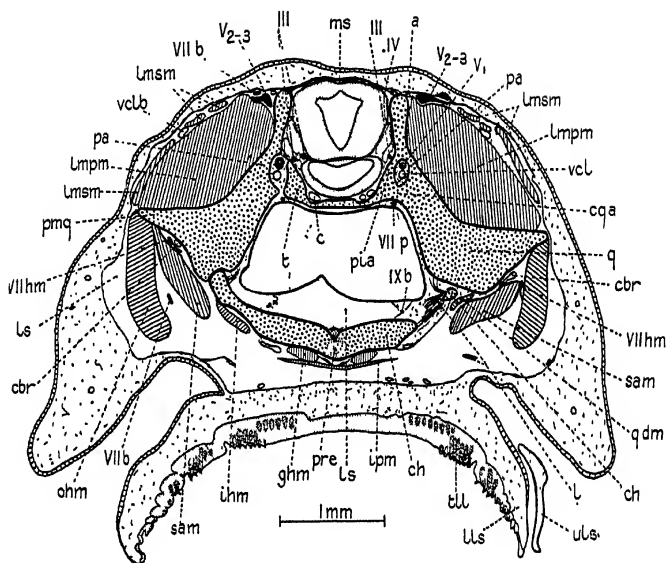
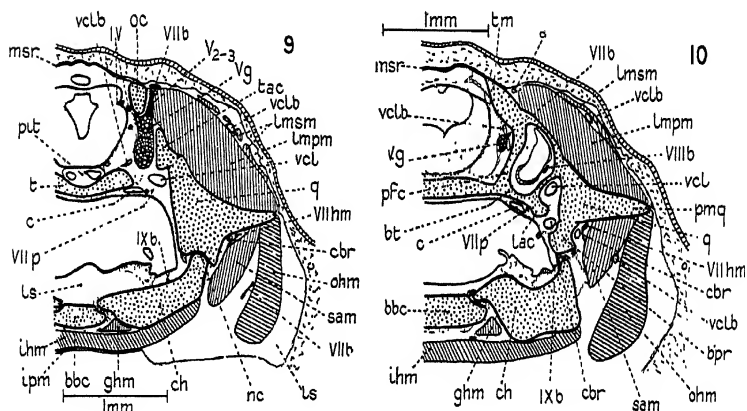
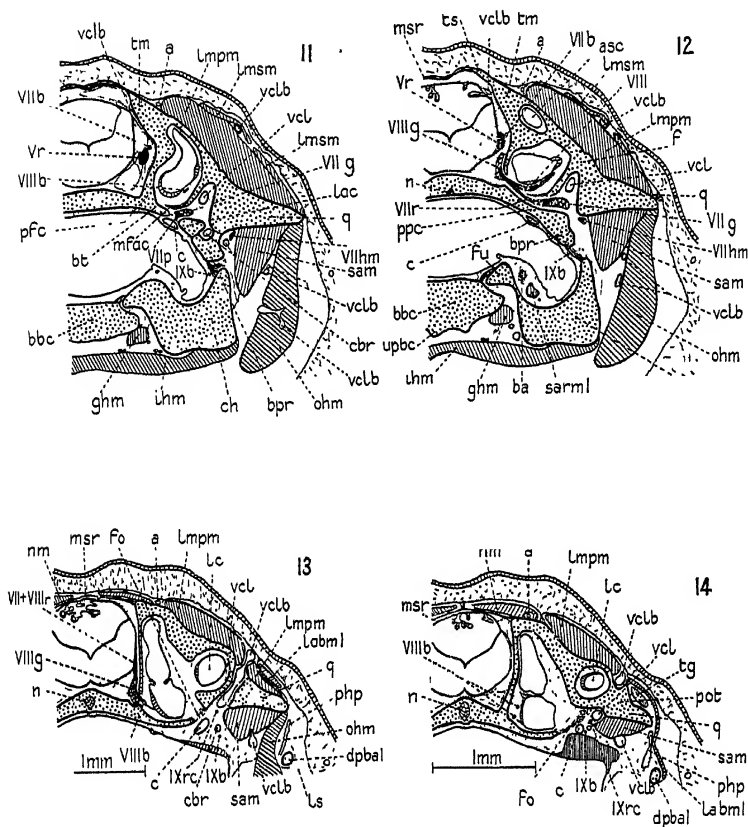
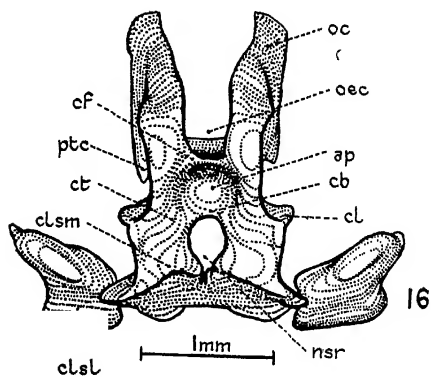
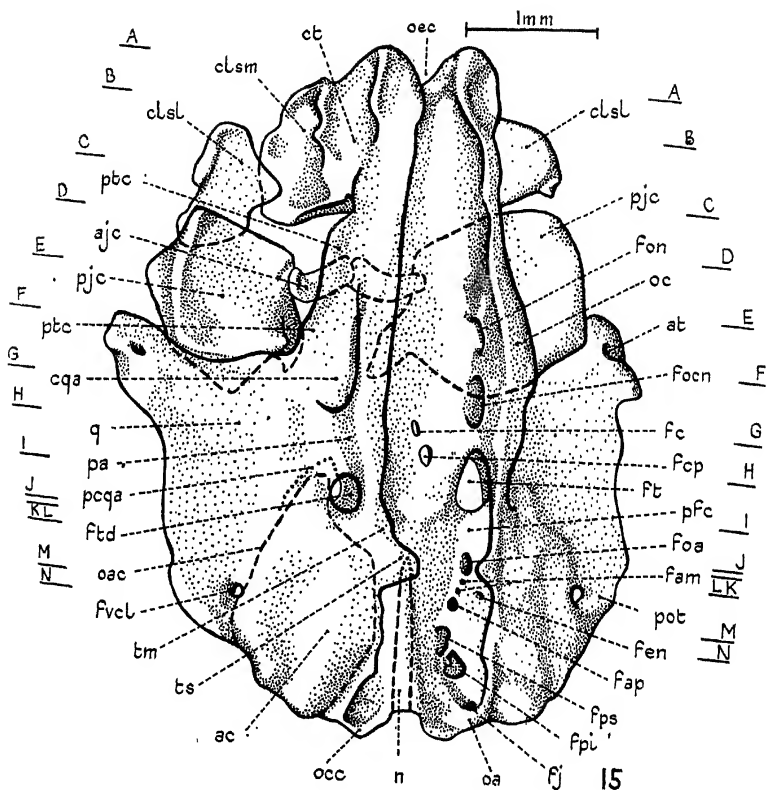


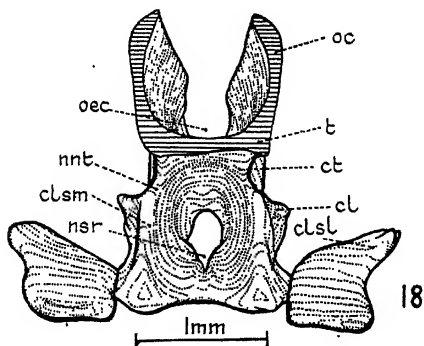
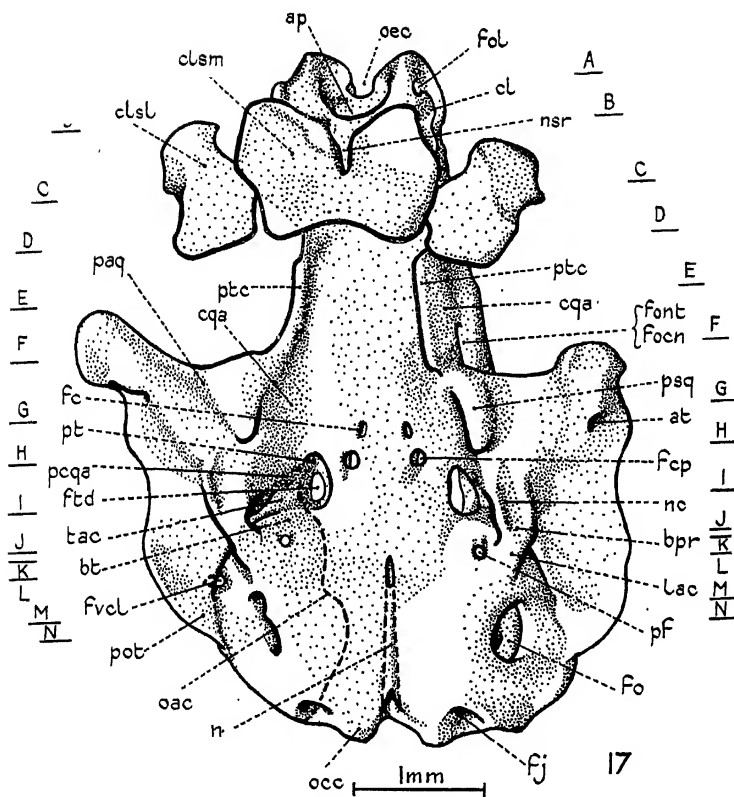
FIG. 8

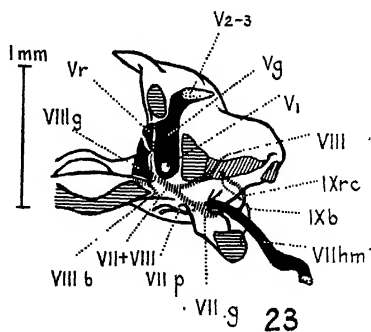
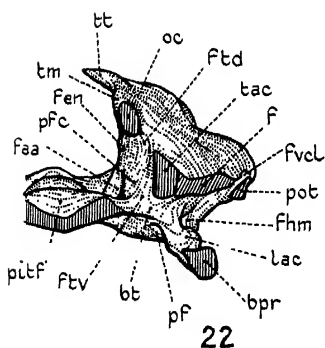
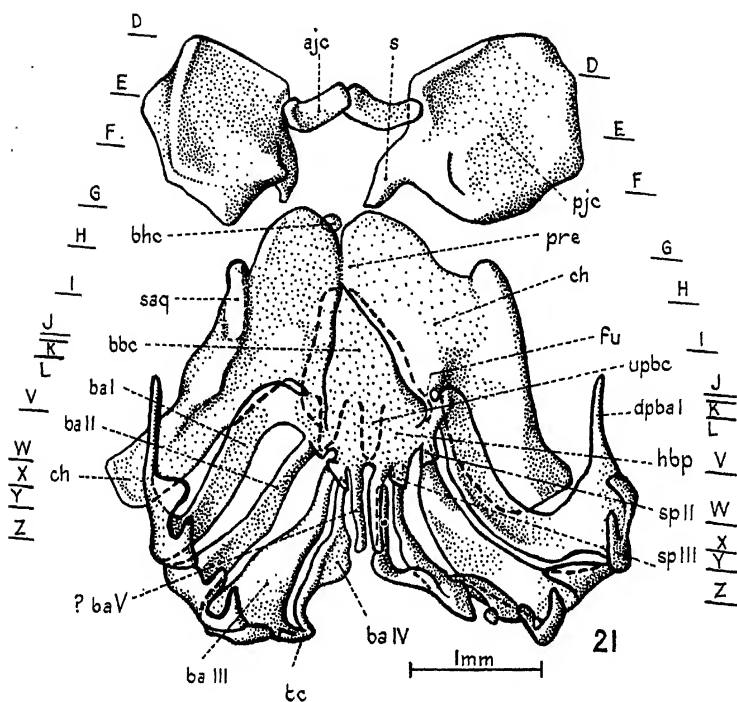




H. K. Pusey del.









On the Ciliary Mechanisms and Interrelationships of Lamellibranchs.

PART VIII: Notes on Gill Musculature in the Microciliobranchia.*

By

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With double Plate 15 and 21 Text-figures.

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* Received August 1939.

INTRODUCTION AND METHODS.

IN the following pages a brief account is given of the musculature responsible for gill movements in the group of Lamelli-branchs for which the name Microciliobranchia has been proposed (Atkins, 1938 *a*). The Microciliobranchia have the latero-frontal tracts of the gill filaments consisting characteristically of a row of micro-latero-frontal cilia, and in this group are placed the Arcacea, Anomiacea, Pteriacea including the Pinnidae, Pectinacea including the Limidae, and the Ostreacea. Gill movements include longitudinal retraction of the gills, approximation and divergence of the demibranchs of a side, dorso-ventral contraction of the demibranchs, and expansion and contraction of the plicae, as well as lesser activities. These movements are not only necessary for the protection of the gills but are closely associated with and essential for the efficient working of the ciliary mechanisms described in previous papers in this series (Atkins, 1936-1938). Slight contractions and expansions of the various gill muscles together effecting dislodgement of particles, opening and closing of grooves, &c., in fact contributing to the efficient working of the ciliary mechanisms, are not necessarily accompanied by closure of the valves. Violent contractions of the chief ctenidial muscles, however, having as their main object the protection of the gills, normally do not occur without strong contraction of the shell muscles and closure of the valves. There is a branchial adductor cycle as described by Janssens (1893) and Setna (1930) in *Pecten* and noted by myself in *Heteranomia* and *Monia* (1936, p. 242) and in *Glycymeris* and *Arca* (p. 221), though in regard to the last two bivalves it was not made clear that contraction of the adductors follows that of the longitudinal muscles of the gill axes. Setna found that, when the branchial nerve or the pallial nerve of the mantle is stimulated, contraction of the gills occurs and subsequently contraction of the adductor muscle. On recovery the adductor relaxes first, followed immediately by the relaxation of the gill muscles.

Most of the gill muscles on violent contraction combine to reduce the size of the gill and to obliterate the interfilamentar

and interlamellar spaces, and so lessen the risk of injury to the gills when water is forced from the shell on sudden closure of the valves.

The Microciliobranchia alone are considered here, but, although many of the group have particularly sensitive and contractile gills, sensitivity is by no means confined to them. Outside the group retraction of the gills has been seen in a number of bivalves, and is especially evident in siphonate forms in which the gills extend into the siphons and suffer retraction together with these: sections, cut for other purposes, have shown longitudinal muscle to be present in the gill axes of *Barnea parva*, *Lutraria lutraria*, *Cultellus pellucidus*, *Ensis siliqua*, *Solen marginatus*, and to be especially abundant in *Solecurtus scopula*. Movements of approximation and divergence of the demibranchs of a side have been observed, for instance in *Modiolus modiolus* and *Mytilus edulis* (Atkins, 1931): dorso-ventral contraction of the demibranchs occurs in a number of bivalves, for instance in *Solen marginatus*, *Hiatella arctica*, *Hiatella rugosa*, *Gastrochaena dubia*, *Barnea parva*, and *Pholadidea loscombiana*: and opening and constriction of the plicae occurs in many bivalves. The pumping movements of the gills of the Protobranchs, *Yoldia* (Drew, 1899) and *Nuculana*, in which striated muscle-fibres are concerned, have been noted in a previous paper (Atkins, 1936).

The sources of the material have already been acknowledged (Atkins, 1938 *a*). For the distribution of muscle-fibres lamellae and individual filaments were examined both living and, after fixation, unstained in glycerine. Numerous sections were prepared: the stains chiefly employed were Mallory's triple stain, eosin and light green (Gatenby, 1928, p. 432), and Heidenhain's iron alum haematoxylin. In sections of the lamellae the main difficulty is to distinguish between fibrous chitin and muscle-fibres. With Mallory's triple stain fibrous chitin and muscle-fibres are clearly distinguished, for chitin, whether pale or deeply staining, becomes blue, while muscle becomes bright red. Eosin and light green is also fairly good, the chitin staining green and muscle pink. With Heidenhain's iron haematoxylin,

in well differentiated sections, the two elements can generally be distinguished for muscle has considerably the greater affinity for this stain; the difficulty is that fibrous, pale-staining chitin then shows faintly. Even the darkly-staining chitin often, though not always, parts with the stain more readily than does muscle.

For the detection of striated muscle-fibre Heidenhain's iron haematoxylin was used on sections, and teased-out fibres from preserved material were examined in glycerine.

All figures were drawn with the aid of a camera lucida.

The researches on which this series of papers, Parts I to VIII,¹ 1936 to 1943, are based were carried out at the Plymouth Marine Biological Laboratory between March 1931 and December 1934.

Acknowledgements for help and criticism were made in Part I, p. 182, 1936.

MUSCLES OF THE GILL AXES.

In members of the group Microciliobranchia the gill axes themselves are free posteriorly, although the upper edges of the ascending lamellae may not be so. The free portion is generally, though not always, of greater length in the Pteriacea and Ostreacea than in the Arcidae, Anomiidae, and Pectinacea. In forms in which the gills are in strong ciliary (Pteriacea) or organic (Ostreacea) union with one another and adjacent parts the freedom of the axes is necessarily masked and can only be observed by dissolving the union.

The chief components of the musculature of the axes are certain longitudinal and transverse muscles.

The longitudinal muscles or branchial retractors are generally, perhaps always, in two sets, one extending from one extremity of the gill to the other, inserted on the shell anteriorly, and responsible for effecting retraction of the entire axis; the other present in the free posterior portion, becoming inserted on the shell where the axis becomes attached, and controlling retraction of that part. There are other minor longitudinal muscles in the gill axes and extreme dorsal region of the descending lamellae

¹ Correction. In Part III, 1937 *a*, last line on p. 392 and line 6 on p. 393, for *autonomy* read *autotomy*.

which cannot be gone into in this paper, although reference may be made to those present in the webs connecting the dorsal ends of the filaments of the single lamella of each demibranch of *Heteranomia squamula*. These on contraction cause the filaments to approach one another in series, thus lessening the interfilamentar spaces (Atkins, 1936, Text-fig. 24, pp. 246-7).

The transverse muscles of the gill axes are generally in two sets, one below and one above the chitinous structure arching the axial food groove. These antagonistic muscles are responsible for causing the two demibranchs of a side to move towards and away from one another.

(a) Forms with Gills Free from one another
and from Adjacent Parts.

In a certain number of forms, Arcidae, Pectinacea, and *Monia* (among the Anomiidae), the gills are apparently free from even effective ciliary connexion with one another and with adjacent parts, the division of the mantle cavity into a supra- and infra-branchial chamber being brought about merely by contact. In these forms interlocking cilia are frequently present on the gills though not on the parts they touch: when the two inner demibranchs are in contact by the dorsal edges of their ascending lamellae there is probably weak, easily dissolved, ciliary union. While this condition is found between the outer demibranchs and the mantle in *Placuna* and *Anomia* (Anomiidae), between the inner demibranchs there is a compound organic and slight ciliary junction in the former, and an entirely organic union in the latter.

Heteranomia squamula will be considered here although its demibranchs, each composed of a single lamella, are in ciliary union at their ventral edges inter se and with adjacent parts.

When the gills are free in the mantle chamber—singly, as in the Arcidae, Pectinacea and *Monia*, or united as in *Placuna* and *Anomia*—they are generally borne on more or less deep suspensory membranes. In *Heteranomia*, in which there is strong ciliary union not only between the gills themselves but between the gills and mantle, the suspensory membrane is narrow dorso-ventrally.

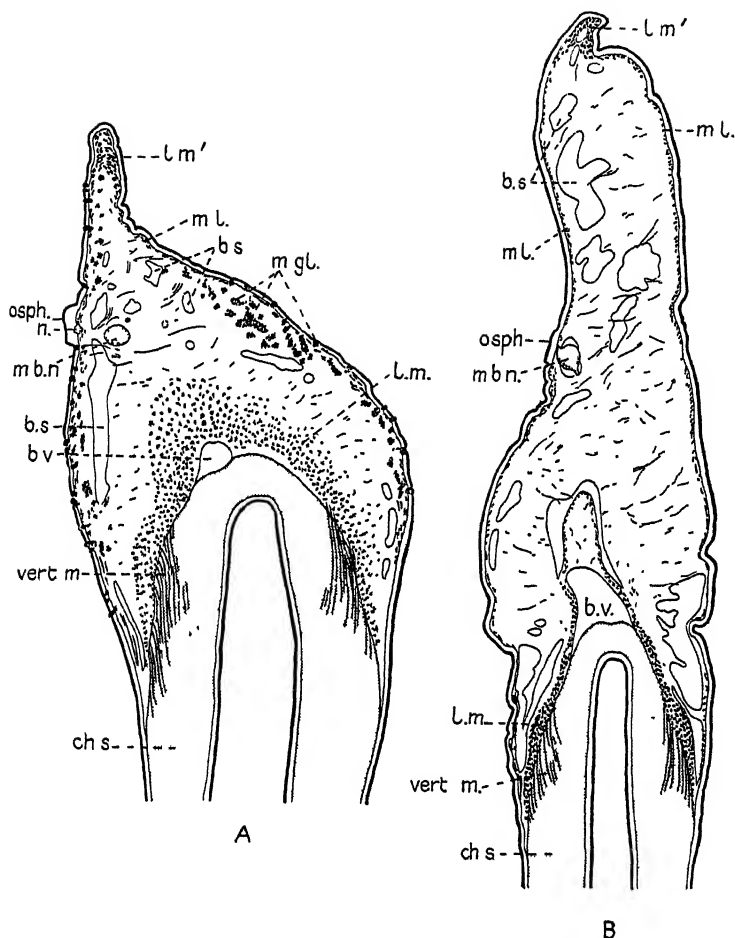
The suspensory membrane is built up of connective and muscular tissue and contains many blood spaces.

Longitudinal Muscles.

A retraction of the gill axis, brought about by contraction of the longitudinal muscles and resulting in the antero-posterior shortening of the entire gill, especially of the free posterior portion, though occurring in all or most members of the Microciliobranchia, is the most obvious movement in those in which the gills are apparently free from even ciliary connexion with the mantle. When asiphonate bivalves are feeding undisturbed, the gill tips and mantle margins extend to or beyond the shell edge, and their withdrawal from this position preparatory to the closing of the valves—whether for the ejection of waste matter, for swimming, as a reaction to much sediment in the water, or on the approach of danger—prevents their being caught by the edges of the shell and injured. As a consequence of the shortening of the axis the filaments are crushed against one another and the lamellar surface may be thrown into uneven vertical folds. This occurs in all the bivalves considered here, being especially marked in swimming forms, in which the gills and highly specialized mantle margins are drawn well away from the shell edge preparatory to the rapid and powerful clapping of the valves in swimming. In all forms, swimming and non-swimming, as a result of the approximation of the filaments and obliteration of the interfilamentar spaces the inhalent current will be reduced or stopped. This may be the reaction of the animal to excessive turbidity and otherwise noxious water.

Arcidae.—The most conspicuous muscles are longitudinal ones running from one end of the suspensory membrane to the other and inserted on the shell anteriorly. In *Arca tetragona* the fibres (*lm*, Text-fig. 1 A) are distributed in transverse section in a wide arc about the dorsal ends of the chitinous supporting structure, in *Glycymeris glycymeris* they form an inverted V, but concentrated towards the two ends to form lateral muscles (*lm*, Text-fig. 1 B), which run across the dorsal ends of the descending filaments.

These longitudinal muscles are responsible for effecting



TEXT-FIG. 1.

Transverse section of the free portion of the suspensory membrane of the gill of, A, *Arca tetragona*, and B, *Glycymeris glycymeris*. *bs*, blood space; *bv*, blood vessel; *chs*, chitinous supporting structure; *lm*, longitudinal muscles; *lm'*, longitudinal muscle in dorsal region of suspensory membrane; *mgl*, mucous glands; *mbn*, main branchial nerve; *ml*, muscular layer beneath epithelium; *n*, nerve; *osph*, osphradium; *vertm*, vertical muscle-fibres. Bouin-Duboscq's fixative (formula given in Atkins, 1937 *b*. p. 424): A. muchamatin and aqueous eosin; B. Mallory's triple stain. $\times 18\frac{1}{2}$. The chitinous supporting structure is shown stippled in this and all figures.

retraction of the gill: on stimulation the gill axes in both bivalves may contract to about three-quarters of their original length, the lamellae being thrown into uneven folds, and the inter-filamentar spaces obliterated. Slight contraction of these muscles causes a gentle swaying of the whole gill anteriorly and then posteriorly.

A diagonally cross-hatched appearance of the muscle-fibres in *Arca tetragona*—*Glycymeris* was not examined for this—is probably due to a spiral arrangement of the fibrils of which they are composed: this is a structure apparently similar to that found in the quick component of the adductor muscles of a number of bivalves, for instance *Anodonta*, *Ostrea*, and others (Marceau, 1909, p. 353; 1936, p. 947). Such spirally striated fibres were found in the ventral or lower portions of the longitudinal muscles; it is not known whether all the fibres have this structure.

Beneath the epithelium in the free portion of the suspensory membrane in both *Arca* and *Glycymeris* is a thin layer of muscle (*ml*, Text-fig. 1), the fibres of which run more or less transverse to the axis in the ventral region, but dorsally become longitudinal in direction. At the free dorsal edge longitudinal muscle-fibres (*lm'*) run from the posterior extremity of the gill to insertion on the shell below the posterior adductor muscle. These muscle-fibres together control dorsalward retraction of the free region, and probably also the slow continuous dorso-ventral movements of the free extremity of the gill seen in the living animal.

In *Glycymeris glycymeris* mucous glands are few in the muscular suspensory membrane, at least in the free portion, the only part sectioned. On the other hand, the suspensory membrane of *Arca tetragona* is provided with numerous mucous glands; an attempt has been made to indicate these in Text-fig. 1 A (*mg**l*). In the attached part of the axis they are especially numerous and form a layer more than 300 μ deep. The puffy, greyish appearance of the membrane in life is probably due to the abundance of these glands. Whether they correspond to the 'white folds' of the mantle of *Monia* is uncertain. It is not known whether in *Arca tetragona* the glandular tissue

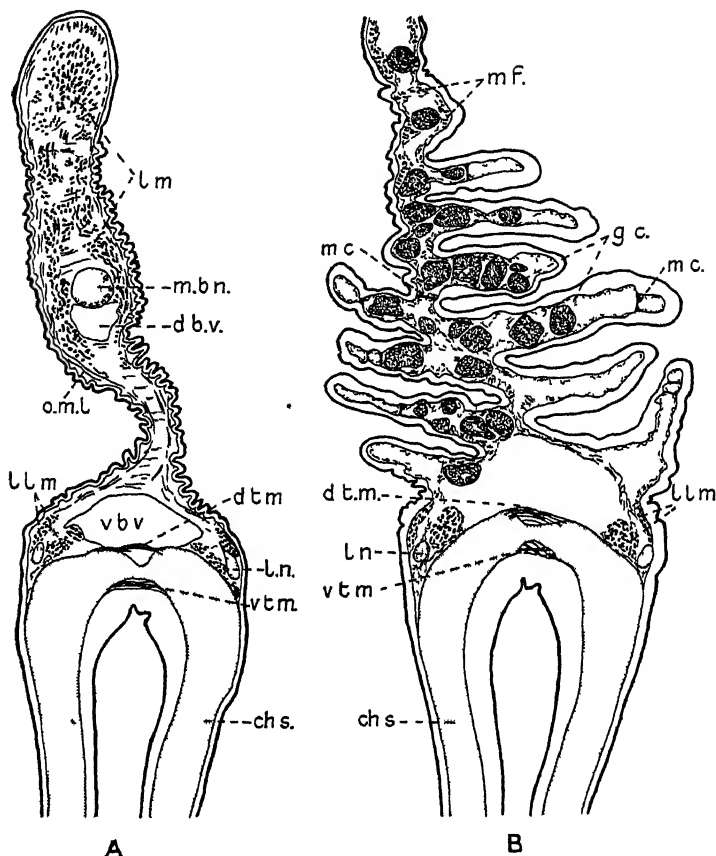
of the suspensory membrane is continued on to the mantle: in *Monia* glandular tissue is present in the mantle above the line of attachment of the suspensory membrane which it does not invade.

Anomiidae.—In *Anomia ephippium* and *Placuna placenta*¹ the deep suspensory membrane in the attached region is thin, and largely occupied by blood spaces. Just above the dorsal ends of the filaments is a row of what appear to be respiratory folds of the membrane. In *Placuna* they run at right angles to the length of the axis, and are about 1.5 mm. long: in my specimens of *Anomia ephippium* contraction of the suspensory membrane, both antero-posteriorly and dorso-ventrally, on fixation has contorted the folds, so that they are cut obliquely in the section shown in Text-fig. 2 B. These folds in *Anomia* and *Placuna* are of the suspensory membrane itself; the 'white folds' (hypobranchial gland) in *Monia* (Atkins, 1936) are glandular folds of the mantle above the line of junction of the suspensory membrane to which they are more or less parallel.

Transverse sections of the attached portion of the suspensory membrane of *Anomia ephippium* show rounded groups of granular cells (*gc*, Text-fig. 2 B). It was suggested in a previous paper (Atkins, 1936) that these might be glandular, but I now think this is at least doubtful, though fixation is not sufficiently good for the histology to be made out clearly. These bodies are in the blood spaces and are present not only in the suspensory membrane, but also in a row along the dorsal edges of the ascending lamellae—where they are sometimes applied to the nerves (Text-fig. 11 D and E, p. 220)—and in the mantle: it is possible that they occur in other parts of the animal. They may possibly be parasites, although it is fairly certain that they are not plasmodia of *Rhopalura*, an *Orthonectid* found in *Heteranomia squamula* (Atkins, 1933).

In the *Anomiidae* (*Heteranomia*, Text-fig. 3; *Monia*, *Anomia*, Text-fig. 2, and *Placuna*) lateral or paired longitudinal muscles are distinct; as two widely separated muscles

¹ According to the label attached to the piece of gill received from the British Museum there is some doubt as to the specific identification.



TEXT-FIG. 2.

Anomia ephippium. Tr. sect. of suspensory membrane of gill, A, in free region, B, in attached region. *chs*, chitinous supporting structure; *dbv*, dorsal blood vessel; *dtm*, dorsal transverse muscle-fibres; *gc*, groups of granular cells; *llm*, lateral or paired longitudinal muscles; *lm*, longitudinal muscle; *ln*, lateral nerve; *mc*, muscle-cell of respiratory folds of suspensory membrane; *mbn*, main branchial nerve; *mf*, muscle-fibres probably responsible for dorso-ventral contraction of membrane; *oml*, outer muscular layer of fibres at right angles to axis; *vbv*, ventral blood vessel; *vtm*, ventral transverse and criss-cross muscle-fibres. The fixation is not sufficiently good to show the osphradium clearly. Bouin-Duboscq's fixative: A, iron haematoxylin and acid fuchsin; B, Mallory's triple stain. $\times 70$.

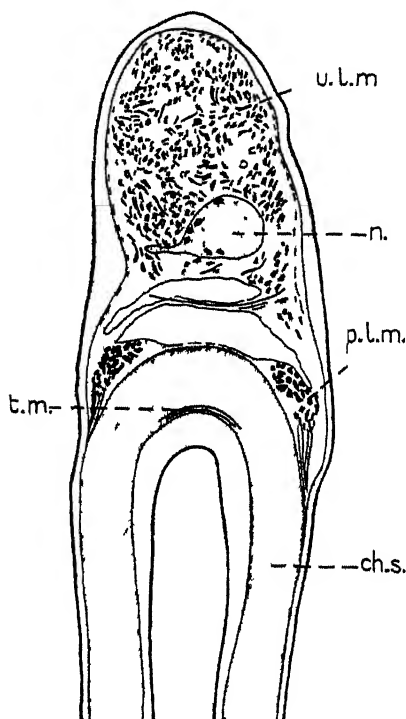
they run the entire length of the gill base, being inserted on the shell anteriorly. In my sections of *Monia patelliformis* and *Monia squama*, though not in those of *Heteranomia*, *Anomia*, and *Placuna*, the lateral muscles are low in position, running across the abfrontal surface of the descending filaments at some little distance below their dorsal ends; this, however, may be due to the much contracted state of the gill after fixation. Hornell (1909, p. 71) has stated that in *Placuna placenta* 'These two muscular cords function as branchial retractors, shortening the gills upon retraction and drawing them inwards, an action in which they are assisted by fine muscle-fibres, which radiate outwards within the branchial mesenteries'. In *Monia* the free extremities of the gills are in continuous movement, moving in the manner of a narrow pointed tongue. On stimulation the gill axes are strongly retracted—the gills being thrown into folds—and the gills, with the ventral edges of the demibranchs touching, bend away from the shell edge.

In *Heteranomia squamula* the lateral or paired muscles (*plm*, Text-fig. 8) consist of two parts, the dorsal fibres being more or less transversely striated (Atkins, 1936, p. 243) and the ventral ones unstriated. The striations are finer than those of the fibres of the lateral muscles of *Pecten* and *Amusium*, but are clearly visible at a magnification of 1470 diameters in sections stained with iron haematoxylin, and also in unstained fibres examined in glycerine at a magnification of 675 diameters. The quick component of the adductor muscle of certain of the Anomiidae is known to be composed of transversely striated fibres (*Anomia*, Marceau, 1909, 1936; *Monia*, Yonge, 1936, Atkins, 1936; and *Heteranomia*, Atkins, 1936), as is also a portion of the byssal muscle of *Heteranomia* and *Monia* (Atkins, 1936, p. 242).

Sections were not cut to investigate whether or no there are striated fibres in the longitudinal muscle of the free region and in the lateral muscles in *Monia*, *Anomia*, and *Placuna*.

In *Heteranomia* the suspensory membrane is considerably less deep dorso-ventrally than in the other genera of the family, and the free posterior portion is largely occupied by

longitudinal muscle, which may be termed the unpaired longitudinal muscle (*ulm*, Text-fig. 3); it runs from the posterior extremity of the gill to insertion on the shell near the adductor



TEXT-FIG. 3.

Heteranomia squamula. Tr. sect. of free portion of gill axis. *chs*, chitinous supporting structure; *n*, main branchial nerve; *plm*, paired or lateral longitudinal muscles; *tm*, transverse muscle-fibres; *ulm*, unpaired longitudinal muscle. Bouin-Duboscq's fixative: iron haematoxylin and acid fuchsin. $\times 166\frac{2}{3}$.

muscle. In those forms in which the suspensory membrane is of considerable depth, i.e. *Anomia* and *Monia*, the longitudinal muscle (*lm*, Text-fig. 2A) of the free portion is less concentrated than in *Heteranomia*. Between the main

longitudinal muscle and the external epithelium is a layer of muscle-fibres at right angles to the axis, and some few fibres of this direction are found in the centre of the suspensory membrane. In *Anomia* (*Aenigma*) *aenigmatica* longitudinal muscle is apparently well developed only on the left side of the animal (Bourne, 1907, pp. 258, 264).

The muscles of the free portion of the axis doubtless assist in the anterior and dorsalward retraction of that part of the gill. In *Heteranomia squamula* some of the fibres of the unpaired longitudinal muscle are striated.

Pectinacea.—The length of the free portion of the axis varies in different forms. According to Pelseneer (1911, p. 29) it is very short in the free and swimming *Pectens*, and long in those with a stout byssus. In *Lima* hians about two-thirds or more of the gill axis is free.

The arrangement of the muscles of the suspensory membrane is closely comparable, though with certain slight differences, in the *Pectinidae*, *Amussiidae*, *Spondylidae* and *Limidae*.

The muscles and nerves of the gill, and especially of the axis, of *Pecten* have attracted the attention of several workers, notably Janssens (1898), Drew (1906), Dakin (1909), and more recently Setna (1930), who along with Drew, apparently did not consult Janssens's paper. Those of *Lima* have been described by Studnitz (1931, pp. 239–41). Janssens described and figured in considerable detail the innervation, circulation, and musculature of the gill of *Pecten* (*jacobaeus*, *varius*, and *maximus*) and noted not only the gill movements brought about by the longitudinal and transverse muscles, but also the role of these and other smaller muscles of the axis in the branchial blood circulation.

In the *Pectinidae*, *Amussiidae*, *Spondylidae*, and *Limidae* lateral or paired longitudinal muscles are present (*Ulm*, Text-fig. 4, p. 204), as in the *Anomiidae*. The course and insertion of these muscles in *Lima inflata* has been described by Studnitz (1931). According to him they arise from the shell orally as a single muscle, which on entering the axis divides into two cords traversing the gill to the posterior extremity. In *Lima* the insertion of this muscle on the shell is in front of the adductor

muscle (see Studnitz, 1931, fig. 19, p. 237): in *Amussium pleuronectes*, *Pecten*, and *Spondylus gaederopus* the insertion is antero-dorsal to the adductor: Pelseneer (1911, pl. xii, fig. 13) showed this muscle in a figure of *Hemipecten forbesianus*.

Janssens (1893) found that in *Pecten* contraction of the lateral branchial muscles diminished the length of the gill nearly to a third—this was also noticed by Setna (1930)—and increased the folding. Studnitz (1931) stated that to these muscles must be ascribed the chief part of the active gill movements in *Lima*.

Janssens in 1893 (p. 32) found very distinctly striated fibres in the lateral muscles of *Pecten jacobaeus*. Dakin in 1909 (1909 a, p. 33) stated that the lateral muscles of *Chlamys opercularis* in certain sections showed 'a very similar striation to that of the pallial muscles' which he had previously discovered to be striated (1909); while in 1930 Setna (p. 377) noted that those of both *Chlamys opercularis* and *Chlamys tigrina* were striated. From my sections it appears as though the lateral muscles of *Pecten maximus* and *Chlamys distorta* are composed of two portions, one consisting of transversely striated fibres and the other of smooth, and it is probable that in no form are they composed of striated fibres only; this may explain Setna's inability to find striations in those of *Pecten maximus*. *Chlamys opercularis* and *tigrina* were not examined for two kinds of fibres.

In *Amussium pleuronectes* transversely striated fibres occur in the lateral muscles; in *Lima hians* also striated fibres are present, though perhaps not as clearly striated as in the other forms mentioned. In *Spondylus gaederopus* and *Spondylus* sp. (from the Great Barrier Reef) striations could not be distinguished, but it is not impossible that they may be found on a more thorough search.

Bivalves with a portion of the branchial retractor muscles composed of transversely striated fibres are mostly either natatory or allies of such forms and perhaps always have the quick component of the shell adductors composed of transversely striated fibres. Retraction of the gills preparatory to the

clapping of the valves in swimming takes place rapidly and rhythmically.

In the free posterior portion of the suspensory membrane of the Pectinacea there is, in addition to the lateral muscles, a muscular layer beneath the epithelium, particularly well developed in *Amusium pleuronectes* and *Lima hians* so that the greater part of the suspensory membrane is occupied by muscle. In *Pecten* the arrangement of the muscle-fibres in an outer and inner layer at right angles to the axis, with a middle longitudinal layer, has been described by Dakin (1909 *a*, pp. 32-8). In *Amusium pleuronectes* the third or inner layer appears to be little developed, or perhaps absent, but it is possible that the development of this layer may vary in different parts of the free posterior portion of the suspensory membrane. The middle layer of longitudinal fibres is the thickest: as may be seen in the living gill, the fibres of which it is composed are longitudinal in direction posteriorly, but as the attached region is approached they become obliquely transverse, and then transverse, or ventro-dorsal, passing to insertion on the shell. In *Lima* they are inserted on the shell behind the adductor muscle (see also Studnitz, 1931, fig. 19, p. 237); in *Pecten*, *Amusium pleuronectes*, and *Spondylus gaederopus* more or less ventral to the adductor. Drew (1906, pl. 4, fig. 7) showed the scars of these muscles as an indistinct line on the valve of *Pecten tenuicostatus*: he (p. 21) noted that although these muscles are inserted on the shell 'the membrane, however, has the appearance of being suspended from the adductor muscle as connective tissue fibres extend along the surface of the muscle and bend it toward the visceral mass'.

The function of these muscles appears to be chiefly to draw the free posterior portion of the gill inwards away from the shell edge, thus assisting the lateral longitudinal muscles. The structure of the fibres of these muscles was not investigated, but it was noticed that in *Chlamys distorta* certain of them appear to be transversely striated, though not as clearly as those of the lateral muscles. Apart from the clapping of the valves in swimming, occasional sudden closure, accompanied by

retraction of the gills, occurs in response to the presence of accumulations of waste material on certain regions of the mantle and effects its ejection. It was suggested by Drew (1906) that the swimming habit has been evolved from such cleansing movements of ejection: this idea was later independently developed by Yonge (1936).

Transverse Muscles.

Transverse muscles are generally present both below and above the arch of the chitinous skeleton. They bring about movements of approximation and divergence of the demibranchs of a gill, so as to effect dislodgement of mucous-entangled material on the gill surface, and even to fling it off the lamella. Such swaying movements have been observed in *Pecten* (Kellogg, 1915; Setna, 1930), in *Ostrea* (Yonge, 1926) and in *Ensis* (Atkins, 1936).

The ventral muscle-fibres are inserted on the frontal surface of the dorsal ends of the chitinous tubes of the descending filaments and cause by their contraction the approximation of the demibranchs, while the dorsal fibres are inserted on the abfrontal surface and cause by their contraction the separation of the demibranchs. The ventral fibres sometimes have a criss-cross arrangement, particularly in the Pectinacea; this is probably a more effective arrangement than when opposite ends of the fibres are inserted at about the same level.

Arcidae.—No transverse muscles, either ventral or dorsal could be found in *Arca tetragona* and *Glycymeris glycymeris*, but strong muscle-fibres (*vertm*, Text-fig. 1, p. 193), which arise from the longitudinal muscles, run for a short distance almost vertically down one side—but whether anterior or posterior was not determined—of the descending filaments, between the surface epithelium and the chitinous tube, on which they become inserted. No doubt contraction and relaxation of these fibres will respectively cause the demibranchs to diverge and approximate.

Anomiidae.—In *Anomia ephippium* (Text-fig. 2 A and B, p. 196) and *Placuna placenta* both ventral (*vtm*) and dorsal (*dtm*) transverse muscle-fibres are present in the

axes. The fibres of the ventral set occasionally cross one another.

In the living gill of *Monia* the free ventral edges of the two demibranchs of a side were seen to touch and diverge. My sections of the gill axis of *Monia* are unfortunately oblique, and while ventral transverse muscles responsible for the shutting movements were identified, it was impossible to distinguish those responsible for divergence.

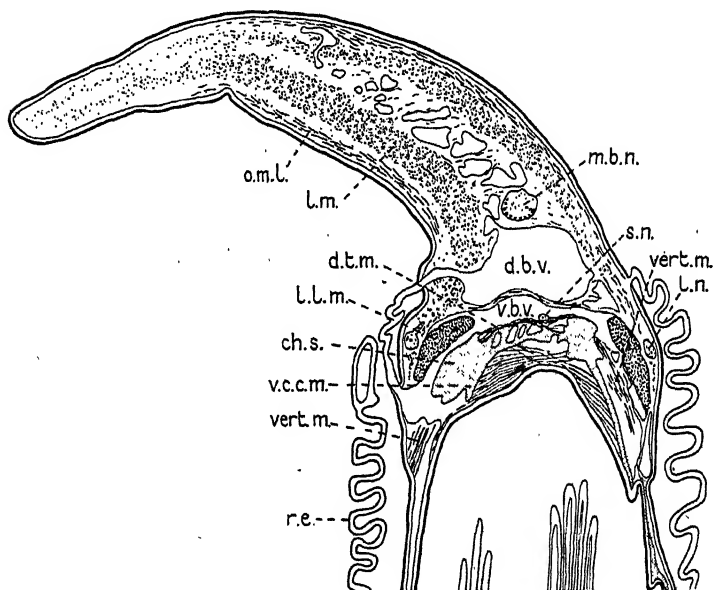
It may be recorded here that the free portion of the gill of *Monia* when removed from the body exhibits movements of approximation and divergence of the demibranchs and shortening of the axis for at least twelve hours after excision. Movements of the excised gill of *Pecten* have been recorded by Drew (1906), Setna (1930), and Gutsell (1931): movement probably continues for a certain time in all lamellibranch gills after excision.

In *Heteranomia squamula*, in which there is strong ciliary connexion of the lower edges of the descending lamellae (ascending lamellae are absent in this bivalve) with adjacent parts, there is little development of muscle-fibres causing movements of approximation and divergence of the demibranchs: some few transverse fibres (*tm*, Text-fig. 3, p. 198) are present below the chitinous arch and some fibres arising from the ventral part of the paired or lateral longitudinal muscles are inserted on the abfrontal surface of the dorsal ends of the chitinous supporting structure of the filaments.

Pectinacea.—In the gill axis of *Pecten* Janssens (1893) has described 'muscles rapprocheurs' and 'muscles écarteurs' and their action in the movements of approximation and divergence of the demibranchs of a gill. These are the muscles later termed 'criss cross' and 'transverse' by Setna (1930, pp. 377-8). Similar muscles occur in the gill axes of *Amusium pleuronectes* (Text-fig. 4, p. 204), and *Spondylus*. In *Lima* hians the fibres of the ventral set appear to be more generally transverse in direction than criss-cross.

The 'criss-cross' muscle-fibres (*vccm*, Text-fig. 4, p. 204) lie below the arch of the chitinous supporting structure and are inserted on the frontal surface of the chitinous tubes of all filaments; the name indicates the manner in which they are

arranged. By their contraction the approximation of the two demibranchs of a gill is effected. Setna (1930, p. 378) found that on the destruction of these fibres with a red-hot needle the demibranchs gaped.



TEXT-FIG. 4.

Amussium pleuronectes. Tr. sect. of free portion of suspensory membrane of gill. *chs*, chitinous supporting structure; *dbv*, dorsal blood vessel; *dtm*, dorsal transverse muscles; *llm*, lateral or paired longitudinal muscles; *lm*, layer of longitudinal muscle; *ln*, lateral nerve; *m.b.n.*, main branchial nerve; *o.m.l.*, outer muscular layer; *re*, respiratory expansion of principal filament; *sn*, subsidiary branchial nerve; *v.b.v.*, ventral blood vessel; *v.c.c.m.*, ventral criss-cross muscles; *vert.m.*, vertical muscle-fibres. Material not sufficiently well preserved to show presence of an osphradium. Alcohol preservation: Mallory's triple stain. $\times 33\frac{1}{2}$.

The transverse muscle-fibres (*dtm*) are situated above the arch of the chitinous supporting structure, and follow a more or less semicircular course, their ends being inserted on the abfrontal surface of the dorsal ends of the chitinous tubes of all

filaments. Contraction of these fibres causes the divergence of the demibranchs.

At their axial ends the principal filaments of the descending lamellae of the two demibranchs of a gill alternate, a principal filament of one descending lamella being opposite an apical filament of the other, and the criss-cross and transverse muscle-fibres which in one lamella are attached to the chitin of a principal filament will in the other be attached to the chitin of an apical and adjacent filaments. The attachment of the muscle-fibres to a principal filament being stronger than to non-principal filaments, the pull is thus equally distributed between the two demibranchs. The alternating arrangement of the plicae of the two demibranchs is probably also the most effective method of packing in a small space the chitinous tubes of the filaments which in plicate gills are much crowded at their axial ends.

(b) Forms with Gills in Ciliary Connexion
with one another and with Adjacent Parts.

In bivalves to be considered here the attachment of the dorsal edges of the ascending lamellae inter se and with adjacent parts is entirely or mainly of a ciliary nature; in *Pinctada*, *Isognomon*, and *Vulsella* between the upturned edges of the inner demibranchs there is in addition to the ciliary junction, organic union, slight in *Pinctada vulgaris* (Herdman, 1905) and *Isognomon alata* (Atkins, 1938 a, pl. 29, fig. 3 B), fairly extensive in *Vulsella* sp. (Text-fig. 11 B, p. 220). Mention of the compound nature of the junction in this undetermined species of *Vulsella* was inadvertently omitted from Part VII, 1938 a.

In members of the Microciliobranchia in which the dorsal edges of the ascending lamellae of the demibranchs are more or less firmly attached to adjacent parts a deep suspensory membrane, such as is present in the Arcidae, Anomiidae, and Pectinacea, is generally absent. In the elongate bivalves, *Pteria*, *Malleus*, *Isognomon*, and *Pinna*, the free posterior portion of the gill axes is very long, in fact in some the axes are free for the greater part of their length, for instance in *Isognomon isognomon* for about three-quarters of the

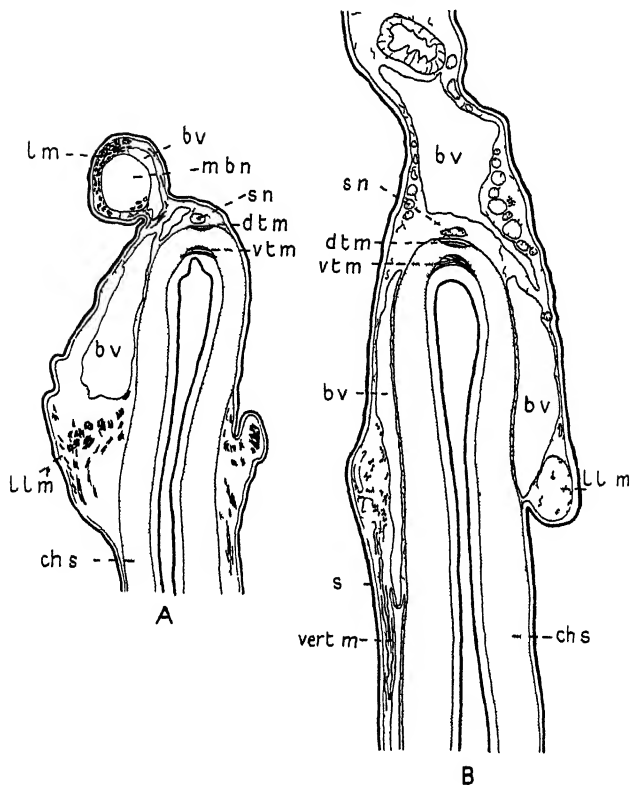
total length, and probably for about the same length in *Malleus albus*, although it is difficult to judge as the body in the specimen examined was much contracted; for about two-thirds in *Pteria hirundo* and for about a half in *Pinctada margaritifera*.

Longitudinal Muscles.

The gills being firmly attached to the mantle, it would seem that retraction of the one must be accompanied by that of the other; in *Pteria hirundo* and *Pinna fragilis* the gills and mantle retract almost to the posterior adductor: Ménégauz (1890, p. 53) mentioned that in *Pinna* there is 'dans les branchies du tissu musculaire qui permet à l'animal de rétracter ces organes avec son manteau'. In a preserved specimen of *Malleus albus* the mantle with the gills firmly attached has retracted to about the middle of the dorso-ventral height of the shell.

The anterior retraction of the gills is due largely to longitudinal muscles in the axes, though generally assisted by muscles in the dorsal edges of the ascending lamellae and ventral edges of the demibranchs. As in the forms in which the gills are free from adjacent parts, certain of these muscles extend from one end of the gill to the other, being inserted on the shell anteriorly. Others are present only in the free posterior portion, passing to insertion on the shell where the axis becomes attached; these latter are particularly abundant in some forms. Perhaps the main objects of the strong retraction of the gills are to remove them from dangerous proximity to the shell edge when the valves are suddenly closed and to prevent excessive fouling of the gills by the sudden ingress of mud or muddy water: gentle muscular movements of the gills assist the ciliary sorting mechanisms.

Pteriidae.—As *Malleus albus* is the only form in which both the free and attached portions of the axis have been sectioned it will be considered first. The gills are long and narrow. Lateral muscles (*Um*, Text-fig. 5 A and B) run from the anterior to the posterior extremity of the gill. They are fairly well developed and low in position, running across the abfrontal surface of the descending filaments at some little distance below

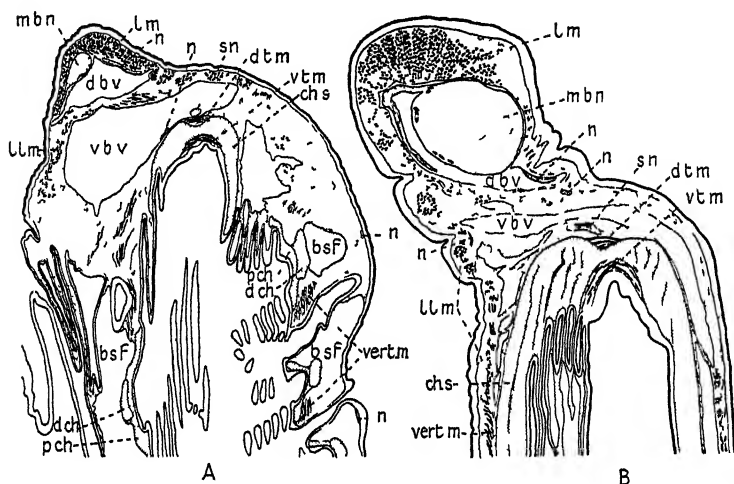


TEXT FIG 5

Malleus albus. Tr sect of gill axis in A free region, and B, attached region. In B only part of the interlamellar septum (s) has been cut as the filament is twisted. *bv*, blood vessel, *chs*, chitinous supporting structure, *dtm*, dorsal transverse muscles, *llm*, lateral or paired longitudinal muscles, *lm*, longitudinal muscle of dorsal region of axis, *mbn*, main branchial nerve, *sn*, subsidiary branchial nerve, *vtm*, ventral transverse and criss cross muscles, *vertm*, vertical muscles of septum bearing filament. Alcohol preservation. A, Mallory's triple stain, B iron haematoxylin and acid fuchsin. $\times 70$

their dorsal ends, in fact they can hardly be said to be in the axis, but are actually in the descending lamellae. It is not known whether this low position is normal, or due to some extent to contraction of the axis on fixation.

In the free posterior region longitudinal muscle (*lm*, Text-fig. 5 A) is present alongside the main branchial nerve (*mbn*) in the dorsal edge of the axis; it passes to insertion on the shell where the axis becomes attached.



TEXT-FIG. 6.

Tr. sect. of free portion of gill axis of A, *Pinctada vulgaris*, and B, *Pteria hirundo*. *bsf*, blood space of principal filament; *chs*, chitinous supporting structure; *dbv*, dorsal blood vessel; *dch*, darkly-staining chitin of principal filament; *dtm*, dorsal transverse muscles; *llm*, lateral longitudinal muscles; *lm*, longitudinal muscle of dorsal region of axis; *mbn*, main branchial nerve; *n*, nerve; *pch*, pale-staining chitin of principal filament; *sn*, subsidiary branchial nerve; *vbv*, ventral blood vessel; *vtm*, ventral transverse and criss-cross muscles; *vertm*, vertical muscles of principal filaments. A, Fixative not known; non haematoxylin; B, Bouin-Duboscq's fixative; Mallory's triple stain A, \times ca. 31; B, \times ca. 43.

In *Pteria hirundo* the muscles (*llm*, Text-fig. 6 B) which correspond to the lateral paired muscles of *Malleus* are in scattered bundles; they appear to be more abundant on one side of the axis than the other. The low position of these muscles is possibly due to contraction of the axis on fixation, as seems to be indicated by the collapsed condition of the main blood vessels.

A great development of longitudinal muscle (*lm*, Text-fig.

6 B) occurs at the free dorsal edge of the axis, partly surrounding the main branchial nerve (*mbn*) which is remarkably large in this bivalve. This muscle corresponds to that in a similar position in *Malleus*: it is inserted on the shell where the axis becomes attached just in front of the adductor muscle, between it and the posterior retractor of the foot.

In *Pinctada vulgaris* (Text-fig. 6 A) the arrangement of the longitudinal muscles is similar to that in *Pteria*, but they are relatively less developed. In my specimen of *Pinctada margaritifera* (the axis was not sectioned) the muscle in the dorsal edge of the free part of the axis appears to run on to the surface of the adductor muscle, but mantle and gills are so contracted that it is difficult to be certain of this.

Vulsellidae.—In *Vulsella* sp. the arrangement of the longitudinal muscles somewhat resembles that of *Pinctada*, but the lateral muscles are little developed.

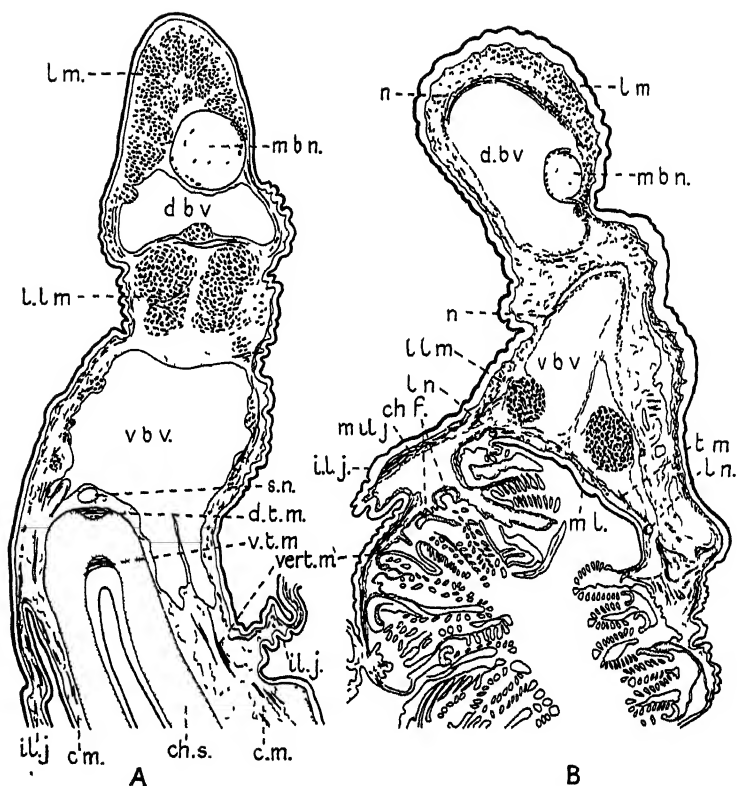
Isognomonidae.—In *Isognomon alata* the large muscles (*llm*, Text-fig. 7 A) which probably correspond to the lateral muscles of *Malleus* are close together, and—perhaps owing to the well distended condition of the main blood vessel above which they run—are in the middle of the axis.

Abundant longitudinal muscle (*lm*) is present in the free dorsal edge of the axis: it passes to insertion on the shell near the anterior end of the almost crescent-shaped adductor muscle.

Pinnidae.—In *Pinna fragilis* the large lateral muscles (*llm*, Text-fig. 7 B) are some little distance apart: they run just above the broad axial food groove.

Other longitudinal muscle is mainly concentrated in a layer (*lm*) at the free dorsal edge of the axis: the axis becomes attached near the posterior adductor muscle when the muscle-fibres pass to insertion on the shell.

Transverse striations could not be recognized in the muscles of the gill axes of *Pteria hirundo*, *Pinctada vulgaris*, and *Malleus albus*; the other forms were not examined for this. It is of interest in this connexion that the fibres of the quick component of the shell adductors of *Pinctada margaritifera* and *Pinna fragilis* have spiral, and not transverse, striations according to Yonge (1936, p. 90).



TEXT-FIG. 7.

Tr. sect. of free portion of gill axis of, A, *Isognomon alata*; B, *Pinna fragilis*. *chf*, chitin of principal filament; *chs*, chitinous supporting structure; *cm*, web connecting upper parts of descending filaments; *dbv*, dorsal blood vessel; *dtm*, dorsal transverse muscles; *ilj*, interlamellar junction; *llm*, lateral or paired longitudinal muscles; *lm*, longitudinal muscle of dorsal region of axis; *ln*, lateral nerve; *mbn*, main branchial nerve; *milj*, muscle passing into interlamellar junction, a small part of which is cut; *ml*, muscular layer of longitudinal fibres above axial food groove; *n*, nerve; *sn*, subsidiary branchial nerve; *tm*, transverse muscles; *vbv*, ventral blood vessel; *vtm*, ventral transverse muscles; *vertm*, vertical muscles of the lamella. A, Alcohol preservation; B, Bouin-Duboscq's fixative; A and B iron haematoxylin and acid fuchsin. A, $\times 70$; B, $\times 18\frac{1}{2}$.

Transverse Muscles.

Ventral and dorsal transverse muscles are present in the gill axes of *Pteria hirundo*, *Pinctada vulgaris*, *Malleus albus*, *Vulsella* sp., and *Isognomon alata* (Text-figs. 5-7), indicating that movements of approximation and divergence of the two demibranchs of a gill occur: they are not, however, as well developed as in the *Pectinacea*.

In *Pinna fragilis* I was unable to discern these two sets of transverse muscle-fibres. Dorsal of a thin layer of longitudinal muscle-fibres (*ml*, Text-fig. 7 B) just above the epithelium of the axial food groove, between the two demibranchs of a gill, are transverse fibres (*tm*), but the course and attachments of these could not be definitely traced in the contracted condition of the axis: certain of them appeared to join with the vertical muscles of the descending lamellae. Whether these transverse muscle-fibres effect movements of the demibranchs, or only contraction of the walls of the dorsal food groove, is not known.

(c) Forms with Gills in Organic Union with one another and with Adjacent Parts.

Longitudinal Muscles.

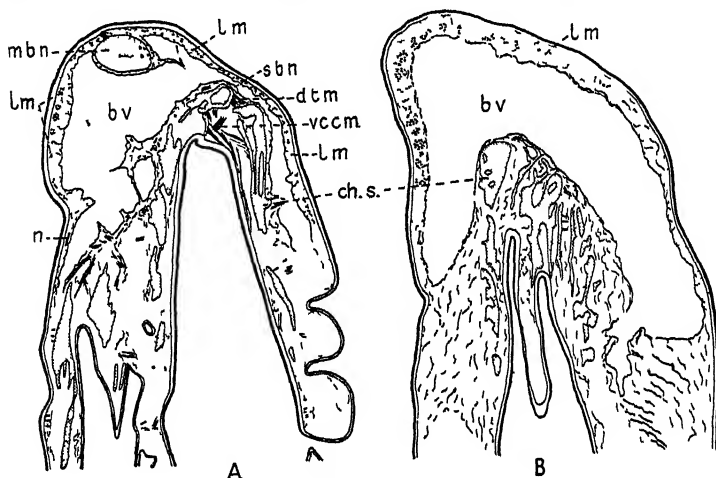
The only family of the group *Microciliobranchia* with entirely organic union between the gills and adjacent parts is the *Ostreidae*. The axis—there is no deep suspensory membrane—is free for about three-quarters of its length. In *Ostrea edulis*, the only species examined for muscle, longitudinal muscle (*lm*, Text-fig. 8 A) is not abundant and in the free portion of the axis—the only part sectioned—is mostly restricted to somewhat meagre bundles beneath the epithelium.

Pelseneer (1911, p. 27) mentioned an anterior retractor of the gill observable in certain species of *Ostrea* on the left side. The scar of this muscle is visible on both valves of *Ostrea edulis*.

Transverse Muscles.

Ventral criss-cross and dorsal transverse muscle-fibres are present (Text-fig. 8 A), indicating that movements of approximation and divergence of the two demibranchs of a gill occur.

Yonge (1926, p. 325) has noted such swaying movements of the demibranchs, together with opening and contraction of the grooves, and the part they play in the sorting of material.



TEXT-FIG. 8.

Ostrea edulis. Tr. sect. of, A, free portion of gill axis, B, union of dorsal edges of ascending lamellae of inner demibranchs in the same region (section somewhat oblique). *bv*, blood vessel; *chs*, chitinous supporting structure; *dtm*, dorsal transverse muscles; *lm*, longitudinal muscle; *mbn*, main branchial nerve; *n*, nerve; *sbn*, subsidiary branchial nerve; *vccm*, ventral criss-cross muscles. Bouin-Duboscq's fixative; iron haematoxylin and acid fuchsin. $\times 56$.

MUSCLES OF THE DEMIBRANCHS.

Our knowledge of the musculature of the demibranchs of members of the group Microciliobranchia¹ is not great, though

¹ References to muscles in the demibranchs of bivalves other than Microciliobranchia are scattered in the literature. It is outside the scope of this paper to give them here, but mention may be made of the interesting discovery of a rich development of muscle-fibres in the interlamellar junctions of the marsupial gills of various Unionidae by Lefevre and Curtis ("Studies on the Reproduction and Artificial Propagation of Fresh-water Mussels", 'Bull. Bur. Fish.', vol. xxx, 1910, issued 1912) and by Bloomer ("Notes on the Anatomy of some African Naiades, Parts I and II", 'Proc. Malac. Soc.', vol. xx, 1932-3; "Note on the Sex of *Anodonta anatina*", *ibid.*, vol. xxii, 1936).

accounts have been written of that of the gill axes. Drew (1906) described in the gills of *Pecten tenuicostatus* muscle-fibres of the interlamellar junctions serving to draw the two lamellae of a demibranch together, and also muscles of the organic interfilamentar junctions serving to draw the filaments together and so shorten the gill antero-posteriorly. Kellogg (1915), on the other hand, denied the presence of muscle-fibres in the gill of *Pecten*, while yet describing violent muscular movement 'which results in spreading open and then constricting the grooves'. Janssens (1893, pl. iii, figs. 86, 91) showed, but did not describe, horizontal fibres in the principal filaments of *Pecten* which he labelled 'fibres élastiques ou musculaires'. Setna (1930, p. 379) stated of *Pecten* only that 'in addition to these muscles (i.e. those of the gill axes) there are other muscle-fibres which run into the interfilamentar junctions, and also throughout the length of the principal filaments, and it is due to these that the principal filaments exhibit activity': he figured them in his fig. 2, pl. 16.

Grave (1911) mentioned muscle strands in the gills of *Atrina rigida*.

Ridewood (1903) in his monograph on the gills of the Lamelli-branchia made occasional mention of the presence of muscle-fibres in the gills, but the stain he chiefly used—borax carmine with picro-nigrosin—is evidently not good for distinguishing between fibrous chitin and muscle-fibres, for he mentioned of *Ostrea edulis* that 'There is a fair amount of muscle in the interfilamentar junctions and in the inner edge of the horizontal septa, but it becomes very difficult in places to discriminate between muscle fibre and fibrous chitin'. Apart from *Ostrea edulis*, of the bivalves considered in the present paper he mentioned that in *Malleus albus* and in *Pinna* there is a longitudinal muscle in the ventral edge of the demibranch, and that in the latter bivalve 'muscle strands run in the upper and lower edges of the interlamellar bands, and are continued upward and downward along the interlamellar edges of the principal filaments for some distance'. He figured (1903, fig. 16, p. 212), though he did not describe, vertical muscles in the principal filaments of *Avicula* (= *Pteria*) *argentea*.

MUSCLES OF THE EDGES OF THE DEMIBRANCHS.

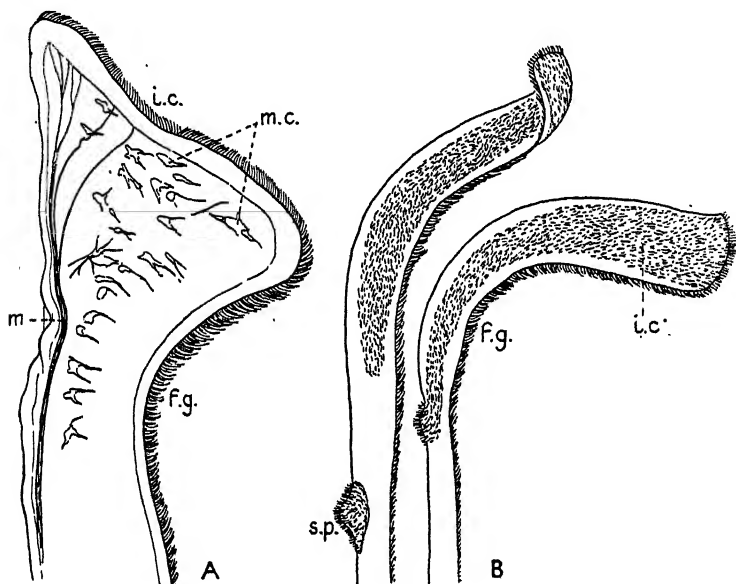
(a) Muscles controlling Movements of the Walls of the Food Grooves.

The importance in certain Lamellibranchs of movements of the walls of the marginal food grooves, those at the ventral edges of the demibranchs, in the sorting of material was mentioned in a previous paper (Atkins, 1937). In a number of the Microciliobranchia, namely the Arcidae, Anomiidae, and Pectinacea including the Limidae, the ventral edges of the demibranchs are ungrooved or only very slightly grooved, but in others marginal grooves are present and play an important part in the sorting of material.

It was found very difficult to discover muscle-fibres likely to be responsible for effecting movements of the walls of the marginal grooves; I was unable to find them in *Ostrea edulis*, *Pteria hirundo*, and *Malleus albus*, but this is no doubt due to their fineness and scarcity, and in some instances to poor preservation of material. In *Pinna fragilis* in which the marginal groove is large, fine semicircular muscle-fibres were discerned beneath the epithelium of the groove (the magnification of Text-fig. 11 F, p. 220, is too low for these to be shown); no doubt contraction and relaxation of these fibres will respectively effect the closing and opening of the groove. No extensor muscles of the groove could be found.

The food grooves at the bases of the gills undergo widening and narrowing. For the axial grooves between the descending lamellae this will be effected by the dorsal and ventral transverse muscles of the axis, which are also responsible for movements of divergence and approximation of the demibranchs of a gill (see pp. 202, 211). Alteration in the curvature of the grooves along the dorsal edges of the ascending lamellae is due to the action of the muscles in the edges of these lamellae. Not much attention has been paid to the latter muscles, but it may be mentioned that in certain bivalves in which the curved dorsal ends of the ascending filaments are in ciliary connexion only, these ends are capable of considerable movement, as those of *Monia* (Atkins, 1936, p. 249), and especially those of *Pecten* when separated

from one another. The ends, which are normally hooked, can become almost straight (Text-fig. 9), and may even be bent backwards. In *Pecten* the muscles bringing about these movements are found spread out in the abfrontal portion of the head of the filament (*m*, Text-fig. 9 A). They are probably not



TEXT-FIG. 9.

- A, Dorsal end of ascending filament of *Chlamys vitrea* unstained in glycerine. Interlocking cilia (*ic*) are shown only at edge of filament, $\times 253\frac{1}{2}$. B, Sketches of dorsal ends of living filaments of *Pecten sp.*, to show change in curvature, $\times 57\frac{1}{2}$. *fg*, food groove; *ic*, interlocking cilia; *m*, muscle responsible for movement of end of filament; *mc*, muscle cells serving to contract filament antero-posteriorly, seen optically in more or less transverse section; *sp*, spur bearing interlocking cilia.

only concerned in altering the curvature of the food groove, but may also play some part in approximating and separating the upturned edges of the two inner demibranchs, and that of the outer demibranch and the mantle with which there is no ciliary connexion.

In *Anomia ephippium* in which the dorsal ends of the

ascending filaments are fused in series, obliquely transverse muscle-fibres (*tm*, Text-fig. 11 D, E, p. 220) are present in the dorsal edges of the ascending lamellae. In the conjoined edges of the ascending lamellae of the inner demibranchs (Text-fig. 11 D) the sets of fibres corresponding to the two lamellae have become more or less continuous; in some sections their inner ends are inserted medianly in close apposition on the chitinous skeleton, while in others the fibres appear to be continuous: the outer ends of the more oblique fibres are inserted on the abfrontal surface of the dorsal ends of the chitinous tube of the filaments, while those of the more transverse fibres are embedded in the somewhat vesicular pale-staining chitin beneath the epithelium. Although living *Anomia* were not seen, it is fairly certain that contraction of these muscle-fibres will result in the separation of the inner lamellae of the two inner demibranchs and in the widening of the food groove between them. In the dorsal edges of the ascending lamellae of the outer demibranchs (Text-fig. 11 E) there are also obliquely transverse muscle-fibres, here inserted mainly on the abfrontal face of the skeleton of the ascending filaments and of the velar filaments (the long ventrally reflected dorsal ends of the ascending filaments of the outer demibranchs, characteristic of the genera *Anomia* and *Placuna*), although some are embedded in the pale-staining chitin underlying the external epithelium. Contraction of these transverse fibres will no doubt effect the separation of the ascending and velar filaments and the widening of the food groove between them. It will also bring the velar filaments against the mantle, with which there is apparently no ciliary connexion, for although interlocking cilia are present on the velar filaments (*ic*, Text-fig. 11 E) there appear to be none on the mantle (see Bourne, 1907, on *Anomia* (*Aenigma*) *aenigmatica*).

A similar arrangement of transverse muscle-fibres is found in *Placuna placenta*.

(b) Longitudinal Muscles.

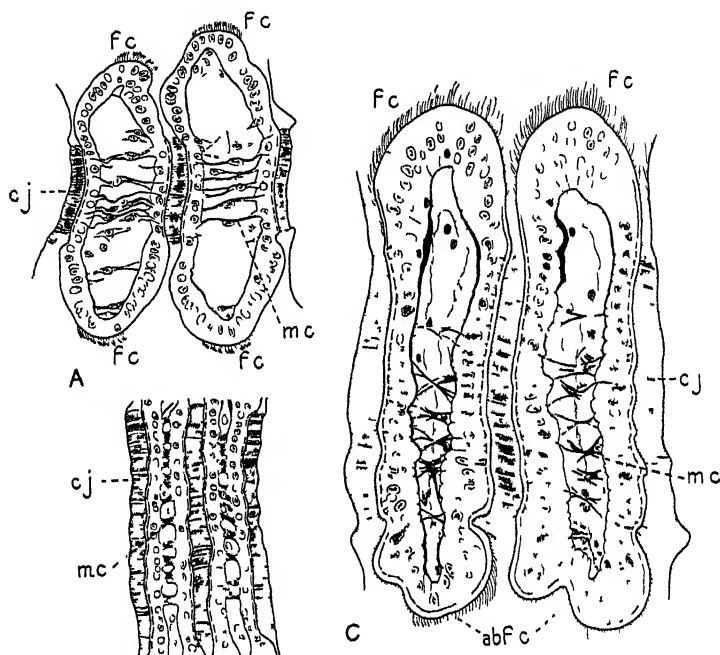
(1) In certain members of the Microciliobranchia with filamentous gills, namely the Arcidae, *Heteranomia* and

Monia among the Anomiidae, the Pectinidae (with the exception of *Pecten tenuicostatus*, see Drew, 1906), Amussiidae, and Spondylidae the filaments at the ventral edges of the demibranchs are connected only by ciliary junctions, as are also the dorsal ends of the ascending filaments, except in *Heteranomia* in which the ascending lamellae are lacking. There are therefore no continuous longitudinal muscles in these positions. In each filament, however, muscle-fibres or cells run between the ciliated disks of opposite sides of the filament (Text-fig. 10). Contraction of these will compress the blood space of the filaments and thus effect contraction of the filaments antero-posteriorly; it is probable that in sympathy the cilia of the disks become more closely interlocked. Thus the free dorsal and ventral edges of the demibranchs of filamentous gills will be shortened longitudinally or antero-posteriorly and reduction of the interfilamentar spaces effected. Nothing has been seen to indicate that the contraction even when strong will cause separation of the interlocking cilia.

It is difficult to trace the insertion of the ends of the muscle cells. In transverse sections of the ventral edge of the demibranch of *Chlamys distorta*, where there is little chitin and the muscles chanced to be in a relaxed state, the muscle-fibres appear actually to enter the cells of the ciliated disks (Text-fig. 10 A). In sections of the ventral edge of the demibranch of *Pecten maximus* when the muscles are strongly contracted the insertion is not clear (Text-fig. 10 B), nor is it altogether clear in transverse sections of the dorsal edge of the ascending lamella of *Arca tetragona* in which the muscle cells are somewhat contracted, and there is a good deal of pale-staining chitin underlying the epithelium of the ciliated junctions (Text-fig. 10 C). It is probable that the fibres actually enter the cells of the ciliated disks, although as pale-staining chitin is laid down more and more thickly the fibres become embedded in it and then lose their connexion with the epithelium.

The muscle-cells crossing the blood spaces of the filaments of *Mytilus edulis* and *Modiolus modiolus* have been described by Lucas (1931, pp. 167-8) as follows:—'Each cell possesses a nucleus. . . . In the cytoplasm of fixed material fine

fibre-like lines occur which vary in size and number and are distributed in all directions within the cell, but their principal



TEXT-FIG. 10.

Tr. sect. through free edges of demibranchs of bivalves with ciliary interfilar junctions in these positions. $\times 342\frac{1}{2}$. A, ventral edge of demibranch of *Chlamys distorta*: muscles relaxed. B, part of section—through ciliary junction—of ventral edge of demibranch of *Pecten maximus*: muscles strongly contracted and interlocking cilia closely interlocked. C, dorsal edge of ascending lamella of *Arca tetragona*: muscles contracted: the frontal portion of filaments cut somewhat obliquely. *abfc*, abfrontal cilia; *cj*, ciliary junction; *fc*, frontal cilia; *mc*, muscle cell. Pale-staining chitin stippled, darkly-staining chitin shown black. At the free edges of the lamellae lateral cilia are absent. Bouin-Duboscq's fixative; iron haematoxylin and acid fuchsin.

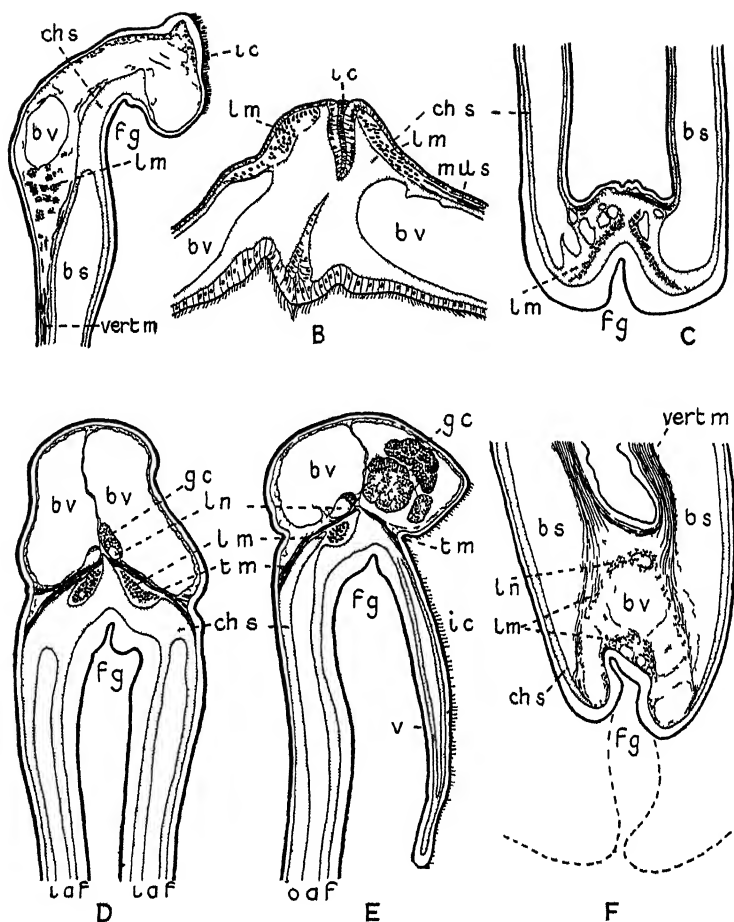
orientation is lengthwise, extending from one chitinous supporting structure to the other. They seem to embed themselves within this chitin,¹ but it is difficult to determine, due to the

¹ This is darkly-staining chitin.

fact that the staining reaction of the fibre which is within the chitin is identical with that of the chitin itself.' In the muscle-cells of the edges of the lamellae of *Arca*, *Pecten*, and others the intracellular fibrils are very clear in fixed material and at the two ends they appear to become distinct from the body of the cell, the cell thus being produced into a dendritic arrangement of fibrils, the ends of which may enter the epithelium of the filaments or become embedded in the underlying chitin. The appearance of the muscle-cells in optical more or less transverse section is seen in Text-fig. 9 A (p. 215). The muscles of the intrafilamentar septum (see p. 251), those of the respiratory folds of the suspensory membrane of *Anomia ephippium* (see *mc*, Text-fig. 2 B, p. 196) and *Placuna placenta*, those running between the ciliated disks of a filament (see p. 234) and also, at least in the smaller species of *Pecten*, the horizontal muscles of the lamellae (see Text-fig. 16, p. 241) have a similar structure.

(2) In certain other members of the Microciliobranchia, including a number in which the gills are termed filibranchiate, the filaments are fused in longitudinal series at the dorsal edges of the ascending lamellae, and in some also at the ventral edges of the demibranchs. Where this occurs longitudinal muscles are present in these positions—generally accompanied by recognizable nerves—and by their contraction effect considerable shortening of the free edges of the demibranchs. The contraction of these muscles probably occurs simultaneously with that of the longitudinal muscles of the gill axes. Anteriorly the insertion of these muscles is on the shell, but it has not been determined whether they have a separate or common insertion, or are inserted on the shell together with the longitudinal muscles of the gill axes as described in *Lima inflata* by Studnitz (1931).

Anomiidae.—In *Anomia ephippium* the filaments are in ciliary connexion at the ventral edge of the demibranch, but the dorsal ends of the ascending filaments are fused in longitudinal series, and behind the visceral mass the upturned edges of the two inner demibranchs are fused *inter se*. In the dorsal edges of the ascending lamellae run longitudinal muscles (*lm*, Text-fig. 11 D and E) whose contraction reduces



TEXT-FIG 11.

Tr sect of dorsal and ventral edges of demibranchs. A, *Malleus albus*, dorsal edge of ascending lamella, $\times 57\frac{1}{2}$. B, *Vulsella* sp., union of dorsal edges of ascending lamellae of inner demibranchs, $\times 140$. C, *Pteria hirundo*, ventral edge of inner demibranch (section passing through ordinary filaments), $\times 95$. D, *Anomia ephippium*, union of dorsal edges of ascending lamellae of inner demibranchs, $\times 70$. E, *Anomia ephippium*, dorsal edge of ascending lamella of outer demibranch, $\times 70$. F, *Pinna fragilis*, ventral edge of outer demibranch (section

the length of the gill. A similar arrangement of the longitudinal muscles is found in *Placuna placenta*.

Limidae.—In *Lima hians* and *Lima loscombi*, which have eulamelliform gills, the upper ends of the ascending filaments are organically united in longitudinal series; the filaments are also in serial organic continuity at the ventral edges of the demibranchs. In both regions longitudinal muscles are present. Studnitz (1931) has described and figured the origin and course of these muscles in *Lima inflata*. According to him the muscle running in the dorsal edge of the outer ascending lamella is inserted on the shell together with the lateral muscles of the gill axis, while that of the dorsal edge of the inner ascending lamella has a separate insertion slightly dorsal to that of the others. The longitudinal muscle running in the ventral edge of each demibranch has no separate insertion, for just in front of the anterior end of the gill each divides out from that of the dorsal edge.

Pteriacea.—In *Pteria hirundo*, *Pinctada vulgaris*, *Pinctada margaritifera*, *Malleus albus*,¹ *Vulsella* sp., *Isognomon isognomon*, *Isognomon alata* (for figure of dorsal edges of inner lamellae see Atkins, 1938 *a*, fig. 3 B, pl. 29), and *Pinna fragilis* the upper ends of the ascending filaments are fused in series, while at the ventral edge of the demibranchs the filaments are also in organic continuity. In both positions generally are found longitudinal

passes through principal filament where marginal food groove has low walls; broken line shows depth of groove in region of apical filaments), $\times 41\frac{1}{2}$. *bs*, blood space of filament; *bv*, blood vessel; *chs*, chitinous supporting structure; *fg*, food groove; *iaf*, inner ascending filament; *ic*, interlocking cilia; *gc*, groups of granular cells; *lm*, longitudinal muscle; *ln*, longitudinal nerve; *mils*, muscles of interlamellar septum; *oaf*, outer ascending filament; *tm*, transverse muscles; *v*, velar fold; *vertm*, vertical muscles of septum-bearing filament. A, B, alcohol preservation; C, D, E, F, Bouin-Duboscq's fixative; A, B, C, iron haematoxylin and acid fuchsin; D, E, Mallory's triple stain; F, iron haematoxylin.

¹ In *Malleus albus* the interfilamentar organic junction at the free ventral edge of the demibranchs is a little distance from the edge, while ventral to it is union by ciliated disks.

muscle and often recognizable nerves (see Text-fig. 11 A, B, C, F). In certain forms, *Malleus albus*, *Pteria hirundo*, *Pinctada vulgaris*, and *Vulsella* sp., muscle is more abundant in the dorsal edge of the ascending lamella than in the ventral edge of the demibranch, but in *Pinna fragilis* it is abundant in both regions. In a preserved specimen of *Pinctada margaritifera* and one of *Isognomon isognomon* the filaments were at an acute angle to the axis, their ventral tips pointing forwards, indicating that retraction of the free ventral edge had been greater than that of the axis.

In *Malleus albus* the longitudinal muscle of the ascending lamella is not at the extreme dorsal edge, but a short distance ventral to it (Text-fig. 11 A): as previously mentioned the lateral longitudinal muscles of the gill axis are also low in position (p. 206).

In the two bivalves, *Malleus albus* and *Pinna fragilis*, in which transverse sections of gill and mantle attached were examined, abundant longitudinal muscle was present in the mantle along the line of ciliary attachment of the gills, indicating that retraction both of gill and mantle takes place along this line. It is probable that muscle is usually present in this position in forms in which the dorsal edges of the ascending lamellae are firmly attached to the mantle by ciliary or organic union, though not in those in which gills and mantle are only in contact.

(c) In the Ostreidae the upper edges of the ascending lamellae of the outer demibranchs are fused with the mantle, those of the inner demibranchs are united in the middle line and fused with the visceral mass anteriorly for a distance varying between about a third and two-thirds of the total length of the gill in different species. In *Ostrea edulis* longitudinal muscle bundles are present in the united dorsal edges of the inner ascending lamellae (*lm*, Text-fig. 8 B, p. 212), being rather more abundant than in the axes. There is some slight development of longitudinal muscle along the line of fusion of the upturned edge of the outer demibranchs with the mantle: there are also some few fibres along the ventral edges of the demibranchs. All these muscles tend to shorten the gill antero-posteriorly on contraction.

VERTICAL MUSCLES OF THE DEMIBRANCHS.

(a) In forms with the gills free in the mantle chamber (Arcidae, Anomidae, Pectinacea) vertical muscles are absent or confined to the dorsal region of the descending lamellae, and there is no shortening or crumpling of the demibranchs, unless it be in Lima by contraction of the longitudinal muscles in the ventral margins of the deep but short (bag-shaped) demibranchs. Where present their function appears to be to effect divergence of the demibranchs of a gill.

In Arca, Glycymeris, and Heteranomia vertical muscle-fibres extending from the longitudinal muscles of the axis a very short distance into the descending filaments are probably responsible for movements of separation of the demibranchs, as already noted (pp. 202-3).

In the Pectinacea also vertical muscle-fibres (*vertm*, Text-fig. 4, p. 204) are present in the dorsal region of the demibranchs. Their axial origin is difficult to determine owing to contraction and contortion of the axis on fixation. Transvers. sections of the suspensory membrane of *Spondylus gaederopus* and *Pecten maximus* clearly reveal that some of the fibres arise from the lateral longitudinal muscles, but it seems that others are inserted on the abfrontal surface of the chitinous skeleton a short distance ventralward of the attachment of the dorsal transverse muscles (*dtm*, Text-fig. 4, p. 204). Vertical muscle runs at first in each intra-plical region, for near the axis the filaments are organically united and there is no intra-plical space. Fibres are given off to all the filaments, though most abundantly to the principal filaments as these become separated from the others. In the filaments they run on each side (anterior and posterior) of the chitinous tube, between it and the surface epithelium, though they are more abundant on one side than on the other. The fibres extend along the principal filaments but a short distance after these become separate, and do not extend as far ventrally in the ordinary as in the principal filaments. They are attached to the chitinous tubes of the filaments.

The function of these short vertical muscles of the Pectinacea would seem to be to effect separation of the demibranchs, in fact to assist the dorsal transverse muscle-fibres of the gill axis.

(b) In certain bivalves having the gills attached to the mantle by strong ciliary junction, namely in *Pteria*, *Pinctada*, *Malleus*, and *Pinna*, a varying, but considerable, degree of dorso-ventral contraction or crumpling of the demibranchs occurs. This has been seen in the living gills of *Pteria hirundo* and *Pinna fragilis*, and may be inferred from the condition of the preserved gills in other forms. Such dorsalward retraction of the demibranchs is due to vertical muscles in the principal filaments and their interlamellar septa in forms with heterorhabdic gills (*Pteria*, *Pinctada*, *Pinna*), and in septa in connexion with ordinary filaments in those with flat, homorhabdic gills (*Malleus*). Although it is in connexion with the septum-bearing filaments that vertical muscles are outstandingly developed, yet vertical fibres do extend for an exceedingly short distance into the dorsal ends of the ordinary filaments where these are fused with one another axially and at the dorsal edges of ascending lamellae. Of the bivalves considered in this paper those with vertical muscle highly developed are in the *Pteriidae* and in the allied *Pinnidae*.

As a general rule the vertical muscles of the descending lamellae arise from the longitudinal muscles of the axis, while those of the ascending lamellae arise from the longitudinal muscles in the dorsal edges of these lamellae. Ventrally some of the fibres join with the longitudinal muscles in the free ventral edge of each demibranch, some are inserted on or embedded in the chitin underlying the epithelium of the marginal food groove or perhaps enter the cells, and some are continuous from the descending to the ascending filaments round the bend of the demibranch (see Text-fig. 11 f).

Contraction of the vertical muscles, reducing the surface of the demibranchs, and, by throwing the filaments against one another obliterating the interfilamentar spaces, and thus decreasing or stopping the inhalent current, may be the reaction of the animal to much sediment in the water entering.

Pteriidae.—In *Pteria hirundo* the course of the vertical muscles has been made out from sections as it was impossible to follow them in entire filaments when contorted by muscular contraction after preservation. Dorsally the majority

of the vertical muscle-fibres of each descending lamella arise from the longitudinal muscles of the axis, while those of the ascending lamellae arise from the longitudinal muscles in the dorsal edges of these. In the dorsal region the vertical muscle runs along one side, but whether anterior or posterior was not determined, of the principal filament, as shown by Ridewood (1903, fig. 16, p. 212) for *Avicula* (= *Pteria*) *argentea*; as the interlamellar septum is approached, however, both the bundle in the descending and that in the ascending arm of the filament divides (fig. 2, pl. 15), one branch continuing along the side (*vertm*¹) and gradually decreasing in size ventrally, while the other (*vertm*²) runs in the abfrontal edge until the septum is reached, when strands from the descending and ascending filaments gradually converge, running closely together (*vertm*, fig. 1, Pl. 15) in the septum to the ventral edge of the demibranch.

In the gill of a young specimen of *Pinctada vulgaris* (25 mm. at the hinge) vertical muscle is present on one side (whether anterior or posterior not determined) of the principal filaments of both descending and ascending lamellae in the dorsal region (fig. 3, Pl. 15): Herdman and Hornell (1904, pl. viii, fig. 13) and Herdman (1905, pl. 27, fig. 8) showed a bundle on each side of the filament; this is possibly due to slight obliquity of the section, so that the inter-spur muscles (see p. 246) are cut more or less transversely. The filaments were much contorted in the ventral region where it was impossible to trace the vertical muscles.

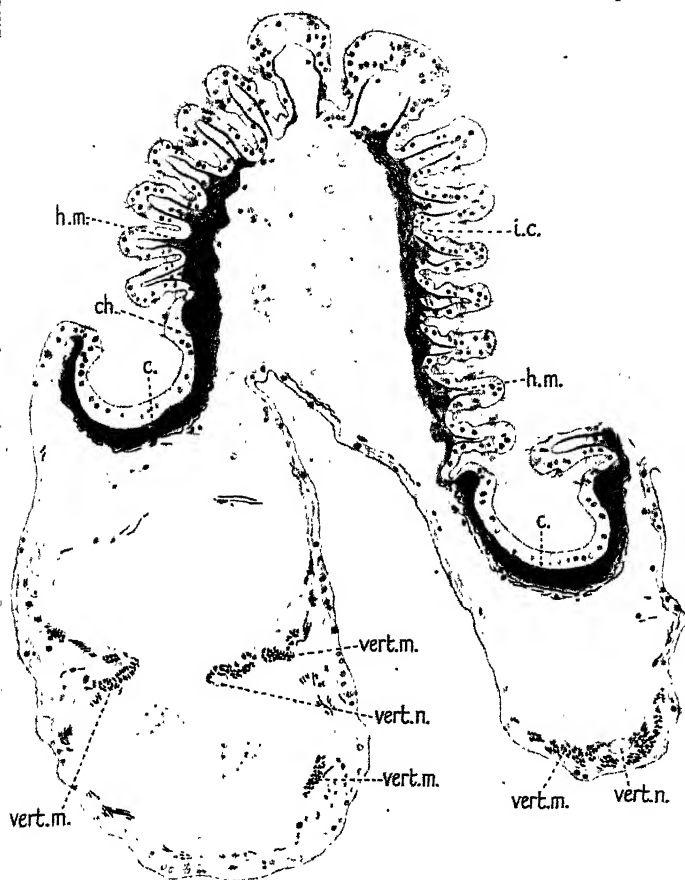
In *Pinctada margaritifera* the vertical muscle (*vertm*, fig. 4, Pl. 15) of the principal filament is abfrontal in position; where the interlamellar septum is present the muscle runs in that. The ascending arm of the principal filament appears to have a lesser development of muscle-fibres than the descending one (contrast figs. 5, 6 with fig. 4, Pl. 15). The distribution of the muscle strands towards the ventral margin of the demibranch was impossible to follow in entire filaments, as in this region the gill was much crumpled owing to the contraction of these muscles.

The dorsal origin of the vertical muscles of *Pinctada*

vulgaris and *Pinctada margaritifera* was not determined, but it is probably the same as in *Pteria*.

Malleus albus with flat, homorhabdic lamellae has interlamellar junctions in the form of septa in connexion with certain of the filaments. According to Ridewood (1903, p. 207) in this species 'every filament has an interlamellar septum, the height of which is very inconstant. About each fourth septum extends the full height of the demibranch.' In a small piece of gill taken from the region where the axis is free and sectioned transverse to the filaments for the present investigation, the number of filaments between septa reaching more or less the full height of the demibranch is five to twelve. These intervening filaments had septa which were little more than the bend of the filament, and can hardly be considered as septa. It may be that the condition of the septa varies in different parts of the demibranch or at different ages. Abundant vertical muscle is present in all the chief interlamellar septa, that is in those extending more or less the full height of the demibranch. Dorsally the vertical muscles of the descending lamellae arise from the lateral longitudinal muscles of the axis, while those of the ascending arise from the longitudinal muscles in the dorsal edge of these. There appears to be a small vertical nerve also in the complete septum, but the material was not well enough preserved for this to be certain. These septum-bearing filaments might perhaps be considered as initial stages in the development of principal filaments. Ridewood (1903, pp. 159-60) rejected the idea because he found that in *Malleus* every filament had a septum of some sort, high, low, or of intermediate extent, and a gill cannot be composed of principal filaments alone.

Pinnidae.—In *Pinna fragilis*, in which extreme dorso-ventral contraction or crumpling of the demibranchs occurs in the living animal on stimulation, vertical muscle strands (*vertm*, Text-fig. 12, p. 227) are abundant in the abfrontal region of both descending and ascending principal filaments, and are accompanied by a nerve (*vertn*). Muscle strands and nerve were shown in a similar position in the principal filaments of *Pinna* (*Atrina*) *rigida* by Grave (1911, fig. 3, p. 420), and muscle



TEXT-FIG. 12.

Pinna fragilis. Tr. sect. of a plica and two principal filaments of one lamella: the principal filament on left shows part of an interlamellar junction. *c*, small canal in chitin; *ch*, chitin; *h.m.*, horizontal muscles; *i.c.*, vestige of ciliary interfilamentar junction; *vert.m.*, vertical muscle; *vert.n.*, vertical nerve. Bouin-Duboscq's fixative; iron haematoxylin and acid fuchsin. $\times 160$.

strands in *Pinna virgata* by Ridewood (1903, fig. 17, p. 214). The only vertical muscles Ridewood mentioned as occurring in the species of *Pinna* (*nobilis*, *nigra*, *virgata*, *zealandica*, and *pectinata* (= *fragilis*)) he examined were continuations of the transverse strands of the interlamellar bands or junctions, which 'are continued upward and downward along the interlamellar edges of the principal filaments for some distance'. Nevertheless in *Pinna fragilis* the vertical muscles are continued throughout the depth of the demibranch. Those in the descending lamella seem to arise partly from the small bundles of longitudinal muscle beneath the lateral epithelium of the axis and partly from the paired lateral muscles. The majority of the fibres of the vertical muscles in the ascending lamellae arise from the longitudinal muscles in the dorsal edge of these. In the ventral margin of the demibranch the fibres of the vertical muscles spread out, some run into the longitudinal muscle present in this region, some are inserted on or embedded in the chitin underlying the epithelium of the lobes of the food groove or perhaps enter the cells, while some of the fibres of the descending lamella are continuous with those of the ascending lamella round the bend of the demibranch (Text-fig. 11 F, p. 220).

Vulsellidae and *Isognomonidae*.—Among the *Pterioacea* investigated *Vulsella* sp., *Isognomon alata*, and *Isognomon isognomon* were alone in having vertical muscles confined to the dorsal region of the lamellae. These muscles presumably effect a limited degree of dorsalward retraction of the demibranchs.

In *Vulsella* sp. vertical muscles, though strong, extend from the gill axis and dorsal edge of the ascending lamellae—from the longitudinal muscles of which they appear to arise—only a short distance into the filaments, though somewhat farther into the principal filaments, along which they run mainly on one side. These muscles are apparently responsible for the crumpling of the dorsal region of the lamellae observed in preserved material.

Isognomon isognomon and *Isognomon alata*, in which the lamellae are flat and homorhabdic and the inter-

lamellar junctions have the form of bars (see p. 231), yet have some development of vertical muscles (*vertm.*, Text-fig. 7 A, p. 210); these extend a short distance into the lamellae, spreading through the continuous membrane (*cm*) which runs across the upper parts of the filaments in both the descending and ascending lamellae.

Ostreacea.—In *Ostrea edulis*, in which the upturned edges of the demibranchs are organically united with one another and with adjacent parts, vertical muscle in the demibranchs is meagre. Yonge (1926, fig. 6, p. 303) described and figured slight development of these fibres in the abfrontal region of the principal filaments. I have been unable to find definite bundles—this perhaps depends on the region sectioned—only scattered fibres beneath the abfrontal epithelium; these are continued in the interlamellar septa. Elsey (1935) could find no conspicuous vertical strands in either *Ostrea gigas* or *lurida*.

MUSCLES OF THE INTERLAMELLAR JUNCTIONS.

The muscles of the interlamellar junctions pass between the descending and ascending filaments, and by their contraction cause the approximation of these filaments and of those connected with them, and thus effect the approximation of the corresponding lamellae of a demibranch and the reduction of the interlamellar space.

In the Arcidae (*Arca tetragona*, *Glycymeris glycymeris*) and Anomiidae (*Monia patelliformis*, *Monia squama*, *Anomia ephippium*, *Placuna placenta*) investigated there are no interlamellar junctions, and they are, of course, absent in *Heteranomia squamula* in which each demibranch consists of a single lamella.

In those Pectinacea with homorhabdic lamellae, *Pecten groenlandicus*, *Amussium dalli*, *meridionale*, and *lucidum*, there are also no interlamellar junctions of any kind (Noman, 1882; Ridewood, 1903), but in those with heterorhabdic gills, for instance *Pecten maximus*, *Chlamys opercularis*, *Chlamys distorta*, *Chlamys tigerina*, *Chlamys vitrea*, *Amussium pleuronectes*, *Spondylus gaederopus*, and *Lima hians*, interlamellar

septa of varying height are present in connexion with the principal filaments. Muscle-fibres are mainly concentrated in the dorsal borders of these septa and are continued a short distance along the abfrontal surface of the filaments. Whether the fibres are inserted on the inner surface of the abfrontal part of the chitinous tube of the filaments or are embedded in it was not determined.

In the Pteriacea the form of the interlamellar junctions varies, but, whether as septa or as bars, muscles are present in them, and serve to draw the two lamellae of a demibranch together. When the bars or edges of the septa are high, then it is probable that the interlamellar muscles arise from the longitudinal muscles in the axis and pass to those in the dorsal edges of the ascending lamellae; when the bars or septa are low, then it is probable that the interlamellar muscles run between the vertical muscles in the descending and ascending lamellae.

The interlamellar junctions have the form of septa in connexion only with the principal filaments in *Pteria hirundo*, *Pinctada vulgaris*, and *Vulsella* sp., but in connexion with the ordinary filaments in *Malleus albus*, in which the gills are flat and homorhabdic: in all these bivalves muscle strands are concentrated in the dorsal edges of the septa. In *Pinctada margaritifera* they are also in the form of septa connected with the principal filaments, but in the words of Ridewood (1903, p. 211) 'many of the high septa in the middle of the length of the gill are incomplete in their middle, so that the thickened upper edge remains as a cross-bar stretching from the upper edge of the ascending lamella to the axis'. In these bars are muscle-fibres—especially numerous in their dorsal and ventral edges—which run from the axis through the bar to the dorsal edge of the ascending lamella. They probably join the longitudinal muscles in these positions, but no sections were made to determine this. A few muscle-fibres are also present in the dorsal edge of the separate lower portion of the septum; they probably join the vertical muscles of the descending and ascending lamellae.

In *Isognomon isognomon* and *Isognomon alata* the upper ends of the descending filaments and also those of the

ascending filaments are connected in series by a continuous interfilamentar membrane or web of some depth. At intervals—of 12, 13, or 14 filaments in *Isognomon isognomon*—interlamellar junctions, which have the forms of bars, connect the webs of the descending and ascending lamellae. In the bars are muscle-fibres. They arise from the longitudinal muscles of the axis and pass through the bars, some joining the longitudinal muscle in the dorsal edge of the ascending lamella and others either entering the cells of the ciliated junction, or being inserted in or on the underlying chitin: which it was could not be determined.

In *Pinna fragilis* the interlamellar junctions have the form of bands, two or three to the height of a demibranch. Some of these junctions are high in position and connect the dorsal edges of the ascending lamellae with the axis (see *ilj*, Text-fig. 7 B, p. 210). Muscle-fibres are present in the dorsal and ventral edges of these bands and are continued upward and downward along the abfrontal edges of the principal filaments for some little distance (see also Ridewood, 1903, p. 215); they probably join the vertical muscles. When the bands are high in position the interlamellar muscles arise from the longitudinal muscles of the axis, and the majority join the longitudinal muscles in the dorsal edge of the ascending lamellae.

In *Ostrea edulis* the interlamellar junctions have the form of septa in which muscle-fibres run between the descending and ascending lamellae of a demibranch.

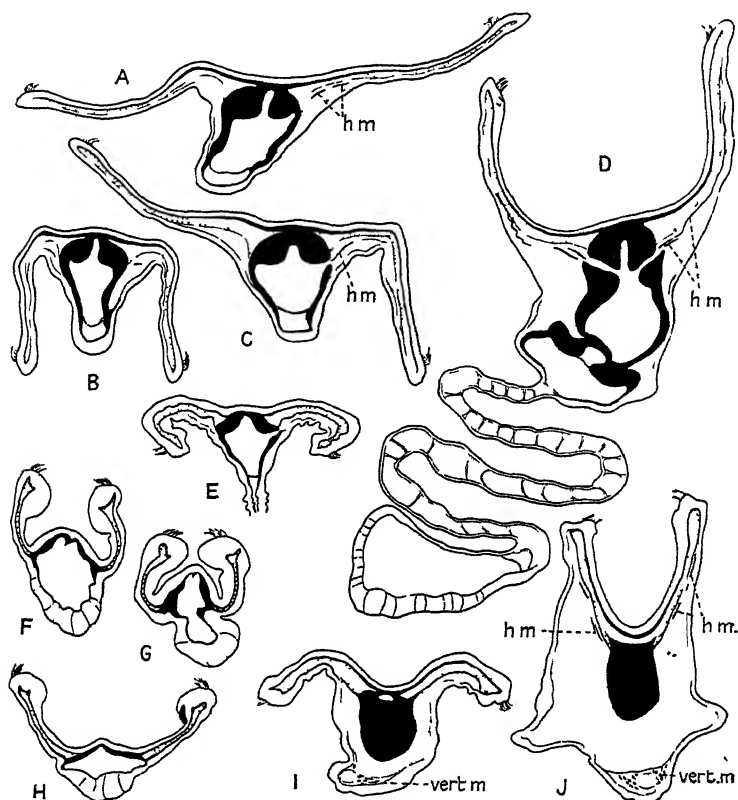
HORIZONTAL MUSCLES OF THE GILL LAMELLAE.

The muscles now to be considered are the horizontal ones running antero-posteriorly chiefly in the principal filaments and responsible not only for changes in the form of the frontal surface of these filaments, but for movements of the plicae. They are longitudinal in direction in relation to the demibranchs, and by their contraction the folding of the demibranch is increased and its length diminished. In those Pectinacea with filamentous gills in which the lateral expansions or wings of the principal filaments are particularly wide, the frontal surface varies greatly in shape according to the state of relaxation and

contraction of the horizontal muscles. The frontal surface of these filaments may be almost flat with the wings more or less in line with the middle part: or deeply grooved, the wings being curved forward; or again, the wings may be bent sharply backward so that they are almost at right angles to the middle of the frontal surface. In the dorsal region of the descending lamellae, where the filaments near their origin from the gill axes are crowded together, movement is restricted, and the principal filaments appear always to have a deeply grooved frontal surface. The movements of the lateral expansions have been aptly likened by Setna (1930) to the flapping movements of a bird's wings. Some sketches of the different shapes assumed by the frontal surface of principal filaments both in bivalves with filamentous and in those with eulamelliform gills are given in Text-fig. 13. Ridewood (1903) does not appear to have fully appreciated the extent to which the shape of the frontal surface of the principal filaments in *Spondylus* and others is dependent on the state of contraction of the horizontal muscles.

The chief development of horizontal muscles is in connexion with the spurs of the filament, which bear the interlocking cilia (ciliary interfilamentar junctions), in forms with filamentous gills, and with the organic interfilamentar junctions in those with eulamelliform gills. These muscles are responsible not only for movements of the principal filaments themselves, but for those of the ordinary filaments attached to them, whether by ciliary or organic junctions. The activity of the lamellae of *Pecten* has long attracted attention (Drew, 1906; Kellogg, 1915; Setna, 1930; Gutsell, 1931), and it was recognized by Kellogg that this activity results in the transference of large or irritating particles—which have stimulated the response—from the principal filaments to the plical crests, along which they are conveyed to the free ventral edge of the demibranch, to be either shaken off on to the mantle and finally rejected, or conveyed towards the palps. Setna (1930) found that the 'curious flapping action' of the principal filaments and of the lamellae generally is due entirely to the muscles of the principal filaments.

Apart from effecting active flapping movements of the principal filaments and of the lamellae, the horizontal muscles



TEXT-FIG. 13.

Sketches to show forms assumed by frontal surface of principal filaments of A-D, *Amussium pleuronectes* (the shape shown in D predominates in dorsal region of demibranch), $\times 223\frac{1}{2}$. E, *Pecten maximus* (from lower part of demibranch), $\times 223\frac{1}{2}$. F-H, *Lima loscombi* (the three sections are at the same level of demibranch), $\times 302\frac{1}{2}$. I, J, *Pinctada margaritifera*, I, ascending, and J, descending, lamella at same level, $\times 123\frac{1}{2}$. A-E, with filamentous gills; F-J, with eulamelliform gills. hm, horizontal muscles; vertm, vertical muscles. Darkly-staining chitin shown black, pale-staining chitin stippled. Only lateral cilia shown.

running into the ciliated spurs and organic interfilamentar junctions are responsible for the opening and closing of the plical grooves, movements noted by various authors, so that in the face of a heavily laden inhalent current the plical grooves—which convey material mainly destined to be eaten—may be hidden and only the plical crests—which are mainly concerned with rejection—exposed (Atkins, 1937, pp. 341–52). It is probable that in forms with eulamelliform gills, especially the Pinnidae and Ostreidae in which horizontal intraplical septa are present, there is little or no active flapping of the sides of the principal filaments.

In forms with filamentous gills muscle-fibres are present in the ciliated spurs of the principal filaments. These spurs are connected by interlocking cilia with the spurs of the ordinary filaments, in which muscle-fibres are also present (*spm*, figs. 1, 2, Pl. 15). It is probable that the fibres in the spurs of the principal and ordinary filaments contract and relax in sympathy and that concurrently with contraction the cilia of the interfilamentar junctions become more closely interlocked, thus tending to draw the filaments closer together and to increase the folding of the gill. In spurs of ordinary filaments muscle-fibres run between the ciliated disks on opposite sides and on contraction draw them together. In the long spurs of *Amussium pleuronectes* and *Spondylus* in addition to fibres running transversely from one ciliated disk to the other, there are fibres which run from the tip to the base of the spur, and on contraction cause bending or crumpling of the spurs. The insertion of the fine end-fibres is difficult to determine. The fibres can be traced through the chitin to the base of the cells of the ciliated disks, but whether they actually enter the cells or the ends are only embedded in the underlying chitin could not be determined. The chitinous lining of the ordinary filaments is continued into their spurs where it is generally thicker though paler staining. The chitin forms pads beneath the epithelium of the ciliated disks, and frequently the cavity of the spurs is almost filled with it.

It will be best to consider the horizontal muscle-fibres of (a) the Pectinacea, (b) the Pteriacea with filamentous gills, (c) the Pinnidae, and (d) the Ostreacea separately, and, as these muscle-fibres are in intimate relation with the chitinous skeleton, it will

be necessary to preface the accounts with a brief description of the skeleton of the principal filaments, which differs somewhat in the different groups.

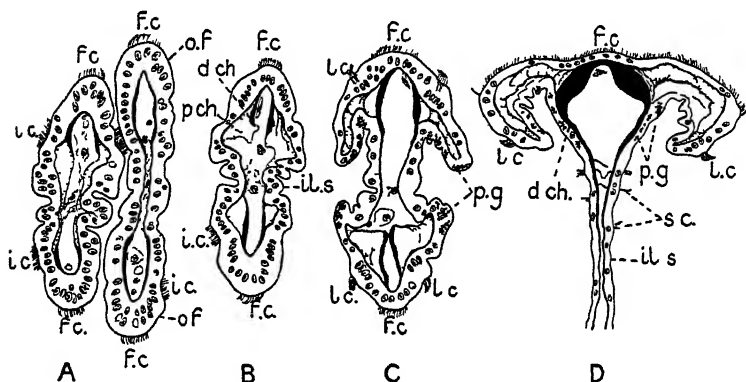
(a) Pectinacea.

Chitinous supporting structure of the principal filaments.—Although the Limidae—with eulamelliform gills—differ in the form of the chitinous skeleton of the principal filaments from those Pectinacea with filamentous gills, yet they largely agree in the arrangement of their horizontal muscles, and so will be considered together.

In the Pectinacea, with the exception of the Limidae, the chitinous skeleton of the principal filaments is of the same general form in all members with such filaments. The main part consists of a more or less complete tube of darkly-staining chitin, compressed laterally, that is antero-posteriorly, and greatly thickened beneath the frontal surface in two longitudinal ridges, with a deep and narrow groove in the middle line (figs. 8 and 9, Pl. 15). These ridges act as pads or cushions over which the horizontal muscles pass. The darkly-staining chitinous tube is present in the thick middle region of the filament and does not extend into the wings. It supports not only the frontal, but also the lateral surfaces of the filament, and in consequence the main blood space is fairly constant in shape in preserved material, differing in this from the Pteriacea with filamentous gills. The tubular part of the skeleton appears to be a thickening and enlargement of that of the ordinary filaments. The change in form of the principal filament and its chitinous skeleton passing from the ventral margin—the youngest part—of the demibranch dorsally is shown in Text-fig. 14. It is possible that somewhat similar changes may take place in the differentiation of principal from ordinary filaments ontogenetically, for it is known that in the spat of *Pecten* and *Ostrea* the gills are homorhabdic (Jackson, 1890; Yonge, 1926).

In the Pectinidae, Amussiidae, Spondylidae, and also in the Limidae the wings of the principal filaments and the spurs are supported by chitin, which is flexible and pale-staining except for a thin darkly-staining layer directly beneath the epithelium.

In the Limidae (*Lima hians* and *Lima loscombi*)—with eulamelliform gills—the main part of the chitinous skeleton of the principal filaments is not as thick as in the Pectinacea with filamentous gills, is not strongly compressed antero-



TEXT-FIG. 14.

Sketches to show change in shape of principal filament, with change in shape of chitinous supporting structure, of *Pecten maximus* from ventral edge—youngest part—of demibranch dorsalward. For comparison ordinary filaments (*of*) have been included in the first sketch: this is through the bend at the ventral edge, and thus ordinary, as well as principal filaments, of descending and ascending lamellae are connected. A, most ventral, and D, most dorsal section. The lower principal filament in A, B, and C is less developed than the upper one, and probably belongs to the ascending lamella. *dch*, darkly-staining chitin; *fc*, frontal cilia; *ic*, interlocking cilia; *ils*, interlamellar septum; *lc*, lateral cilia; *pch*, pale-staining chitin; *pg*, orange pigment granules; *sc*, sensory cilia. Bouin-Duboscq's fixative; iron haematoxylin and acid fuchsin. $\times 342\frac{1}{2}$.

posteriorly, and supports a greater width of the frontal surface. The abfrontal portion of the principal filament of these two species of *Lima* is much swollen: a condition partly due to the large lumen and partly to the height of the tall mucous cells that compose the abfrontal epithelium. The chitin underlying these gland cells is no more than a pale-staining film affording little support, and in consequence in preserved material the shape of the abfrontal portion of the filament varies considerably in

different regions of the same filament and in different filaments (figs. 10 and 11, Pl. 15).

A curious modification of the darkly-staining chitin of the principal filaments is met with in these Lima (see fig. 12, Pl. 15). The part lying beneath the frontal epithelium is medianly for short distances alternately greatly attenuated—for lengths of about 10 to 18 μ —(fig. 10, Pl. 15) and greatly thickened—for lengths of about 20 to 40 μ —(fig. 11, Pl. 15). In the latter condition the frontal surface is supported by a thick, though narrowly cleft, median ridge of chitin with a low, broad ridge on each side. In the cleft runs what appears to be a broad strand of material which stains black with Heidenhain's iron haematoxylin and bright red with Mallory's triple stain, as do muscle-fibres, but I have been unable to entirely satisfy myself that it is muscle: the appearance may possibly be due to some deposit on the surface of the chitin facing the cleft. The functional significance of the curious alternation of structure of the chitinous skeleton of the principal filaments of Lima is unknown, but it may possibly permit easy puckering of the filaments, which no doubt occurs on contraction of the longitudinal muscles in the ventral edges of the short, but deep, bag-shaped gills (see p. 221).

It has happened that in figs. 10 and 11, Pl. 15, a ridge arising from the bottom of the grooved frontal surface is shown with one condition of the chitinous skeleton, and a flat-bottomed groove with the other. The shape, however, depends on the state of contraction of the horizontal muscles, and not on the underlying chitin, for in sections following that shown in fig. 10, Pl. 15, the frontal groove remains with a ridge arising from the bottom, though the form of the skeleton alternates. Although at the same level of the lamella the wide frontal groove may be either flat-bottomed, or with a ridge arising from the middle, yet, in my sections, the flat-bottomed condition is more usual in the dorsal region of the demibranch, and the ridged condition in the ventral region.

The darkly-staining chitinous tube of the principal filaments of the Pectinacea is interrupted at short intervals for the passage of the muscles which run across the filament from one margin to

the other, and between the spurs of opposite sides, or, in the case of the Limidae, through the organic interfilamentar junctions. Except in *Pecten maximus* (Text-fig. 17) the horizontal muscle-fibres seem rarely to be inserted on the chitinous tube but pass through (Text-figs. 15, 18).

Both in the Pectinacea and Pteriacea in the wings of the principal filaments and in the spurs the horizontal muscles tend to keep to the abfrontal side of the pale-staining chitin, running between it and the epithelium of the under surface of the wings and spurs (see figs. 3, 5, 7, 9, Pl. 15), although frequently sections reveal fibres surrounded by pale-staining chitin (see figs. 4, 10, 11, Pl. 15).

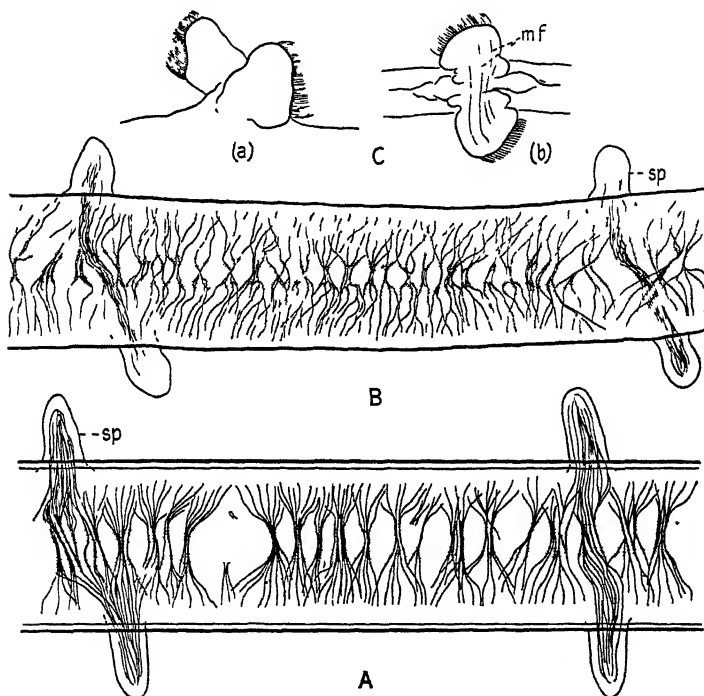
Horizontal Muscles.—The function of these muscles has already been given in the introduction to this section (p. 231).

In the Pectinacea, as in the Pteriacea, the most important muscle-fibres are those in connexion with the spurs. It might be noted here that the spurs of the ordinary filaments of *Chlamys vitrea*¹ are double-headed, one head with its ciliated disk facing anteriorly, and the other with its ciliated disk facing posteriorly (Text-fig. 15 c). In Lima the muscle-fibres which cross the principal filaments in the region of the interfilamentar junctions (corresponding to the spurs of the filibranchiate types) are continued round the abfrontal surface of the plica (Atkins, 1938 a, fig. 1, pl. 29); certain of them are inserted on the inner surface of the abfrontal ends of the chitinous supports of the ordinary filaments. The appearance of the interfilamentar junctions of Lima in the living gill is of fused spurs; there is no organic horizontal septum across the intra-plical space as in Pinna and at intervals in Ostrea (Ridewood, 1903, p. 218), though the two arms of the interfilamentar junctions of a plica are connected by interlocking cilia (Atkins, 1938 a). It has previously been noted (Atkins, 1938 a) that near the free ventral margins—the youngest part—of the demibranch the interfilamentar junctions are of a compound, organic and

¹ The specimen, 15 mm. long and 16 mm. deep, was firmly attached by a well-formed byssus to a worm tube taken from a submarine cable off the west coast of Ireland in lat. 14° W., long. 52° N.

ciliary, nature, indicating the filibranchiate ancestry of the Limidae.

Those horizontal muscles crossing between spurs on opposite



TEXT-FIG. 15.

A, *Chlamys distorta* and B, *Chlamys vitrea*, principal filament from frontal surface to show horizontal muscles, $\times 112$. C, *Chlamys vitrea*, spur of ordinary filament, (a) viewed from the side, but spur somewhat flattened, (b) viewed from abfrontal surface, $\times 344$. mf, muscles; sp, spur bearing interlocking cilia. Chitinous tube shown in optical section and stippled. Unstained in glycerine.

sides of a filament, or from the organic interfilamentar junctions in the Limidae, generally traverse the chitinous tube in so doing. The spurs, however, are not always directly opposite; where the asymmetry is slight some of the fibres are continuous between the two spurs, while others traverse the tube to be inserted on

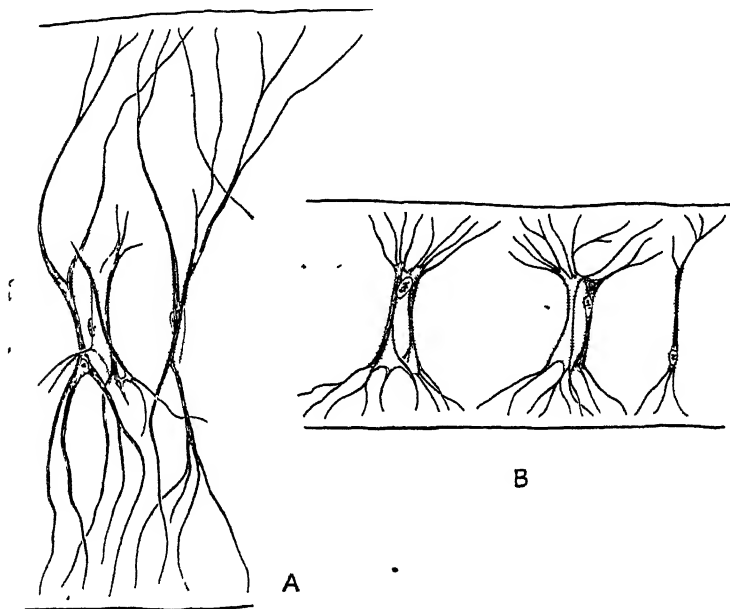
the opposite margin of the filament itself (Text-figs. 15, 18 B), or in *Pecten maximus* are inserted on the chitinous tube without crossing it (Text-fig. 17): where the asymmetry is marked, as frequently in the Spondylidae, practically all the muscle-fibres from a spur are inserted on the opposite margin of the filament (Text-fig. 18 A).

The arrangement of the horizontal muscles is very characteristic in the Pectinacea (see Text-figs. 15, 17, 18), and strikingly similar in such members of the four families as were investigated, although in one the gills are eulamelliform and in the others filamentous. In addition to the muscles which cross between the spurs on opposite sides of the principal filament, there are also groups of muscle-fibres at short intervals which cross from one margin to the other and in so doing traverse the chitinous tube which is interrupted for their passage. Each group of muscle-fibres—and also the inter-spur muscles—has probably originated from one or more muscle-cells similar to those described running between the ciliated disks on opposite sides of the filaments at the ventral and dorsal edges of the lamellae of filamentous gills (see p. 217). This probable origin is seen most clearly in small species of Pectinidae such as *Chlamys vitrea* and *distorta* (see Text-fig. 16). The fibrils or fibres are strongly marked in the muscle-cells; correlated with the development of wings to a principal filament, the branching of the fibres from opposite ends of the muscle-cells is extensive, there being a fan-shaped group at each end of the cell.

In *Pecten maximus* (Text-fig. 17) it appears both from living and preserved material that the body of the muscle-cell is much attenuated, being no more than a fine fibre, and in many instances appears to have atrophied, leaving unconnected fan-shaped groups of fibres on opposite sides of the filament. The inner ends of these groups of fibres appear to have become secondarily inserted in or on the chitinous tube. This is found but rarely in *Chlamys distorta* (Text-fig. 15 A), *Chlamys vitrea* (Text-fig. 15 B), *Chlamys opercularis*, *Chlamys tigrina*, *Amusium pleuronectes* (Text-fig. 18 B), *Spondylus gaederopus*, *Spondylus* sp. (Text-fig. 18 A), and *Lima hians*.

The horizontal muscle-fibres appear to be finer in *Amusium pleuronectes* than in the other Pectinacea, but this may be the result of the imperfect alcohol fixation of museum material.

The insertion of the outer ends of the fan-shaped groups of

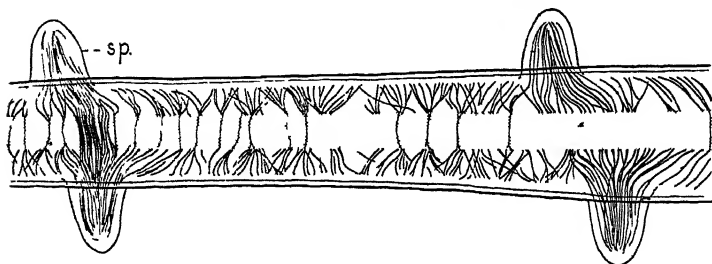


TEXT-FIG. 16.

- A, *Chlamys vitrea*, two groups of horizontal muscles in principal filament near dorsal edge of descending lamella. $\times 274$.
B, *Chlamys distorta*, three groups of horizontal muscles in principal filament towards ventral edge of demibranch where the filaments are narrow. Chitinous tube shown in optical section and stippled. Unstained in glycerine. $\times 274$.

fibres, as well as of the inter-spur muscles, is difficult to make out. The fibres can be traced to the base of the epithelial cells of the margins of the filaments, and in the case of the spur muscles to the base of the cells of the ciliated disks. As in the muscle-cells described on p. 217 the fibres at least in young stages probably enter the cells, but as the deposition of chitin beneath the epithelium proceeds they become embedded in it.

Some variation in the general extent of each fan-shaped group of muscle-fibres has been observed in different forms, but it is not known if this is at all dependent on age or position in the lamella. In *Chlamys opercularis* the fibres spread over a wider area than in *Amussium pleuronectes* and at their outer ends intercross more extensively with those of



TEXT-FIG. 17.

Pecten maximus. Principal filament from frontal surface to show horizontal muscles. *sp*, spur bearing interlocking cilia. Chitinous tube shown in optical section and stippled. Unstained in glycerine. $\times 93\frac{1}{2}$.

adjacent groups; in *Spondylus gaederopus* and *Spondylus* sp. (from the Great Barrier Reef) and in *Lima hians* the groups of muscles are close together and the end branches cover a small area.

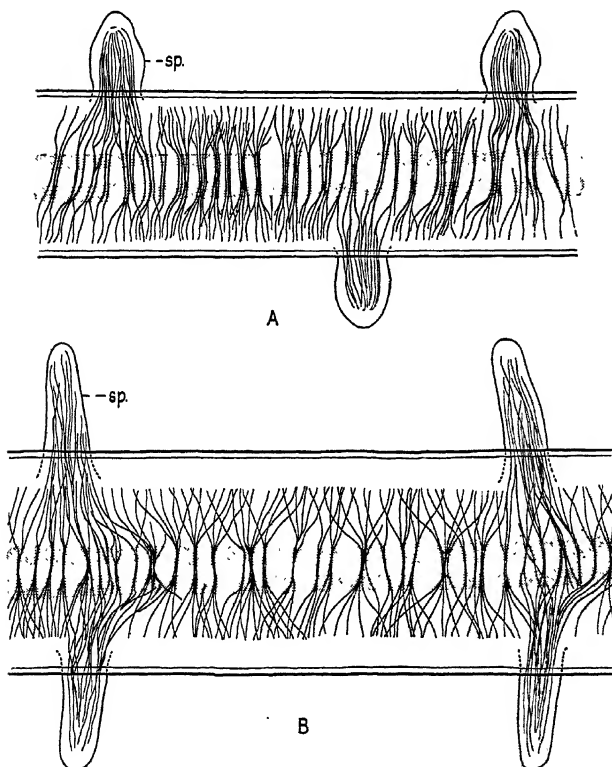
In *Lima hians* and *Lima loscombi*, in addition to the muscles crossing the filament from one margin to the other, there are strong fibres inside the frontal half of the tube having their ends inserted on the inner surface of its side walls: in sections they are frequently seen snapped (figs. 10 and 11, Pl. 15). These fibres by their contraction will draw inwards the side walls of the tube so compressing the blood space, and at the same time will cause the formation of a ridge on the bottom of the grooved frontal surface (fig. 10, Pl. 15).

(b) Pteriacea with Filamentous Gills.

Chitinous supporting structure of principal filaments.—Although in certain of the Pteriacea the inter-

filamentar junctions are of a compound nature, yet the organic part being slight these forms may be considered here.

In *Pteria hirundo* the chitinous skeleton of the principal



TEXT-FIG. 18.

Principal filament from frontal surface of, A, *Spondylus* sp. (from Great Barrier Reef), B, *Amussium pleuronectes* to show horizontal muscles. *sp.*, spur bearing interlocking cilia. Chitinous tube shown in optical section and stippled. Unstained in glycerine. $\times 112$.

filaments differs considerably in the young and adult; it is possible that this is so in other species of *Pteria* and also in

Pinctada. Throughout the demibranch in a young *Pteria hirundo* (23 mm. at the hinge line), but only near the ventral margin—the youngest part—in the adult (fig. 1, Pl. 15) there is little development of darkly-staining chitin: such as there is appears in cross-section as two narrow bars forming an inverted V, the apex being in the middle of the frontal surface. This part of the skeleton seems to correspond to that of the ordinary filaments, the thick layer of pale-staining chitin beneath the frontal surface and extending into the spurs being added during development of the principal filaments, probably by specialization of the ordinary filaments during development, as occurs in *Pecten* (Jackson, 1890). The horizontal muscle-fibres (*hm*, fig. 1, Pl. 15) passing between spurs on opposite sides of a filament run across the inner ends of the Λ -shaped structure, there being no development of solid chitin to the inner side of the muscles. In a well grown *Pteria hirundo* (66 mm. at the hinge line) over most of the lamella, although the Λ -shaped structure is still frequently visible in the thick layer of pale-staining chitin beneath the frontal surface, the space between the arms is now filled in, and additional darkly-staining chitin is present across their inner ends—the skeleton being as shown by Ridewood in *Avicula* (= *Pteria*) *argentea* (1903, fig. 16, p. 212). In the dorsal region of the lamella a massive, somewhat irregular, longitudinal rod of darkly-staining chitin projects into the lumen of the filament (fig. 2, Pl. 15). The skeleton of a principal filament thus differs greatly from that of an ordinary filament. Pale-staining chitin is present beneath the frontal surface, extending into the wings and the spurs, and partly surrounding the rod of chitin (fig. 2, Pl. 15). The abfrontal region of the principal filament is large and quite unsupported by the main skeleton, the chitin underlying the superficial epithelium being no more than a pale-staining film. In consequence, in the preserved gill of *Pteria hirundo* the inner or abfrontal portion of the principal filaments is most irregular and variable in shape: this is found also in *Pinctada* (figs. 3, 6, Pl. 15). The horizontal muscle-fibres still retain the same position relative to the Λ -shaped, now solid triangular, chitinous structure as in the young, passing across its inner

side. They pass between it and the rod of darkly-staining chitin where this is present and appear to be largely surrounded by a pale-staining chitin. This variety is found chiefly in those parts of the filament which undergo considerable movement; it is probably more flexible than the darkly-staining kind, and according to Ridewood (1903, p. 167) it appears to be a softer, less concentrated variety of the normal chitin.

The chitinous supporting structure of the principal filaments of *Pinctada margaritifera*, 52 mm. at the hinge line (fig. 4, Pl. 15), and of *Pinctada vulgaris*, 25 mm. at the hinge line (fig. 3, Pl. 15), is, over the greater part of the lamella, similar to that found in the higher levels of the lamella of *Pteria hirundo* (fig. 2, Pl. 15), while the chitinous skeleton in *Vulsella* sp. of unknown size (fig. 7, Pl. 15) is similar to that in *Pteria hirundo* near the ventral margin of the demibranch (fig. 1, Pl. 15).

Within the pale-staining chitin of most of the bivalves mentioned dark striations are visible. One set is parallel to the frontal surface, the other and darker more or less at right angles to it, and both appear to be growth lines marking successive positions of the external epithelium as the filament widens, for it will be seen that they roughly follow its outline (figs. 1, 2, 4, 7, Pl. 15). These striations seem to indicate that the pale-staining chitin has been laid down by the external epithelium in successive layers. The inverted V-shaped structure was possibly secreted by this epithelium when the principal filaments differed little in shape from ordinary filaments, but the origin of the massive darkly-staining rod of chitin is uncertain. Ridewood (1903, p. 168) discussing the supporting skeleton of the filaments of Lamellibranchs, stated that 'The chitin must be secreted by the cells of the lacunar tissue and not by the superficial epithelium, since it is found in places far removed from the epithelial layer (e.g., in the middle of a filament as an intrafilamentar septum, in the middle of large thick interlamellar septa, and in the principal filaments of *Ostrea*, *Tridacna*, &c.), and on its first formation must be in relation with internal cells, probably disposed with their edges in contact to form an endothelium. In the filaments of the adult, however,

the appearances presented are those of isolated cells or groups of cells flattened against the surface of the chitin.' The intra-filamentar 'septum', however, is not composed of chitin as Kellogg recognized in 1892; he regarded it as endothelial in nature, and it is probably muscular (see p. 251). As for the chitin in the middle of interlamellar septa, it is probable that when first formed the septa were thin, and it is not impossible that they thickened largely as a consequence of successive layers of chitin secreted by the external epithelium. But the formation of the chitinous skeleton of the filaments of Lamellibranchs, as well as the development of the principal filaments, needs investigation.

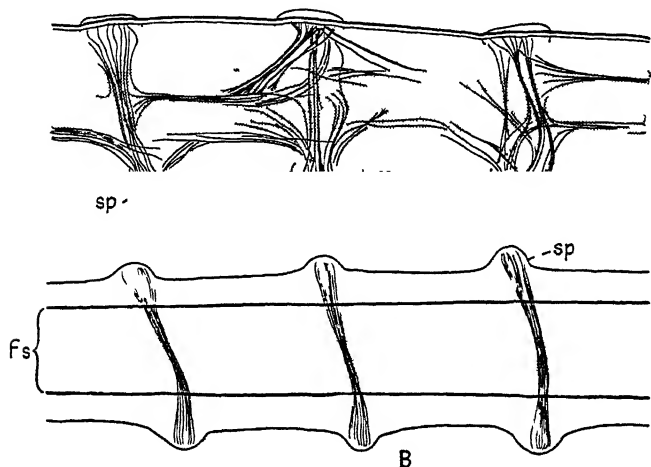
Horizontal muscles.—The most important horizontal muscles are those which run between the spurs of opposite sides of the principal filaments and are responsible not only for movement of these filaments, but also of the ordinary ones attached to them by interlocking cilia, and thus of the lamellae generally (see p. 231): in some forms these are the only horizontal muscles in the principal filaments. Contraction of the horizontal muscles diminishes the length of the demibranch and increases the folding. The muscles assist in the ciliary sorting mechanism, such as described in *Pteria hirundo* (Atkins, 1936, p. 275; 1937, p. 341).

In the Pteriacea, as in the Pectinacea, it could not be determined whether the fibres at the tips of the spurs and at the edges of the filaments actually enter the cells of the external epithelium or are inserted in the chitin underlying it, though fibres were traced to the base of the cells.

In the Pteriidae the disposition of the horizontal muscle-fibres in the principal filaments varies considerably in different forms. In *Pteria hirundo* (Text-fig. 19 A) the arrangement is irregular, especially in the ventral region, compared with that in *Pinctada*. A proportion of the fibres generally run directly between the short, blunt spurs of opposite sides; a number, however, diverge from the direct route and are inserted on the main rod of chitin (fig. 2, Pl. 15); and some few run between adjacent spurs of the same side. These latter fibres are more numerous in that region of the demibranch (Text-fig. 19 A)

where the main branch of the vertical muscle runs in the septum—the branch in the filament itself having disappeared (see p. 225)—than in the dorsal region. The margins of the filaments between the spurs appear to be without muscle-fibres, none crossing the filament.

In the allied genus *Pinctada* the arrangement of the

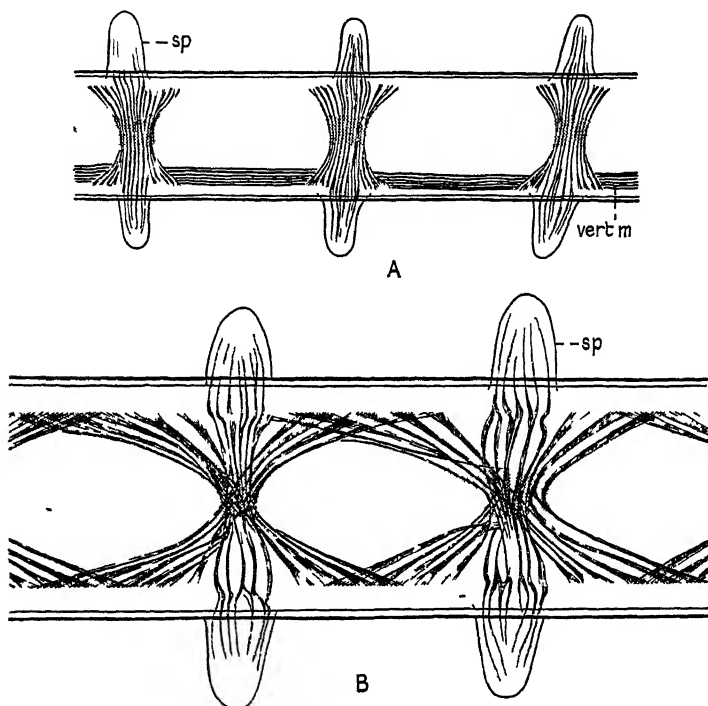


TEXT-FIG. 19.

Principal filament from frontal surface to show horizontal muscles of, A, *Pteria hirundo* (from Naples) in region where vertical muscles mainly run in septum and so are not shown, B, *Vulsella* sp. fs, frontal surface; sp, spur bearing interlocking cilia. Main part of chitinous skeleton, which in *Vulsella* sp. and in all but dorsal part of demibranch of *Pteria hirundo* lies above the horizontal muscles, shown stippled. Unstained in glycerine. $\times 112$.

horizontal muscles is regular. There is a certain difference in the arrangement in the two species, *Pinctada vulgaris* and *margaritifera*, but as the specimens of *Pinctada vulgaris* were small, not more than 25 mm. at the hinge line, while that of *Pinctada margaritifera* was large, 52 mm. along the hinge, it is not impossible that it may be an age difference. In both species the muscles cross the filaments only in the spur region. In *Pinctada vulgaris* (Text-fig. 20 A,

and fig. 3, Pl. 15) in addition to the horizontal muscles running between spurs on opposite sides of the principal filament—which in both species are usually exactly opposite one another—some few fibres on each side of the spur muscles spread out



TEXT-FIG. 20.

Principal filament, seen from frontal surface, to show horizontal muscles of, A, *Pinctada vulgaris*, B, *Pinctada margaritifera*. *sp*, spur bearing interlocking cilia; *vert m*, vertical muscle. Solid chitinous rod, which underlies the horizontal muscles, shown stippled. Unstained in glycerine. A, $\times 202\frac{2}{5}$, B, $\times 112$.

symmetrically and are attached at the two edges, anterior and posterior, of the filament (for insertion see above, p. 246) for a short distance on each side of the spur.

In *Pinctada margaritifera* (Text-fig. 20 B and figs. 4, 6, Pl. 15) the horizontal muscle-fibres form a striking pattern.

Their ends are attached at the sides of the principal filaments over the entire distance between adjacent spurs, the outer ones crossing those from the adjacent group on each side.

Although in both *Pinctada vulgaris* and *Pinctada margaritifera* (figs. 3, 4, Pl. 15; Text-fig. 20) most of the muscle-fibres cross from one side of a principal filament to the other and in so doing pass between—though sometimes surrounded by—the mainly pale-staining chitin underlying the frontal epithelium and the deeply staining longitudinal rod of chitin, which forms the main support of the filament, yet some few fibres are inserted on the sides of the chitinous rod.

From the foregoing account it will be seen that in the Pteriidae, unlike the Pectinacea, muscle-fibres cross the filaments only in the spur regions, and while in *Pinctada*, especially in *Pinctada margaritifera*, certain of these fibres spread out fan-wise to be inserted at the sides of the filaments, in *Pteria hirundo* there is no development of fibres attached at the margins of the filaments between adjacent spurs.

In the Pectinacea the distance between adjacent spurs is generally considerably greater than in the Pteriacea, and this may possibly have some bearing on the different arrangement of the horizontal muscles in the two sub-orders.

In *Vulsella* sp., of the allied family Vulsellidae, the only horizontal muscle-fibres are those passing between spurs of opposite sides (Text-fig. 19 B, p. 247): the size of the specimen from which the fragment of gill was taken is unknown.

(c) Pinnidae.

Chitinous supporting structure of principal filaments.—In *Pinna fragilis* the supporting structure of the semi-cylindrically grooved principal filaments (Text-fig. 12, p. 227) consists of a deep layer of chitin, of fairly even thickness, beneath the frontal epithelium. There is little, if any, difference in the staining intensity of the chitin of the principal filaments and that of the interfilamentar junctions, and in both positions it is fibrous in appearance: the chitinous lining of the ordinary filaments stains rather more darkly.

In the middle of the frontal surface of the principal filament

runs a tiny canal (*c*, Text-fig. 12) immediately below the external epithelium and partly embedded in the chitin: it contains tissue which may be nervous.

Horizontal muscles.—Horizontal muscle-fibres (*hm*) are abundant; they lie below the chitinous layer of the principal filaments and extend into the interfilamentar junctions, passing round the inner surfaces of the plicae, that is, across the inner ends of the ordinary filaments, to the chitin of which some of the fibres are attached. Although the interfilamentar junctions have the form of horizontal septa, horizontal muscle-fibres do not appear to pass directly across the intrapical area, as they do in *Ostrea edulis*. Horizontal muscle-fibres of principal filaments are not confined to regions where interfilamentar junctions occur, but between such regions they pass from one margin to the other, being inserted at the sides of the filaments. As in the other groups, although the horizontal muscles were traced to the base of the cells of the external epithelium, it could not be determined whether they enter these cells.

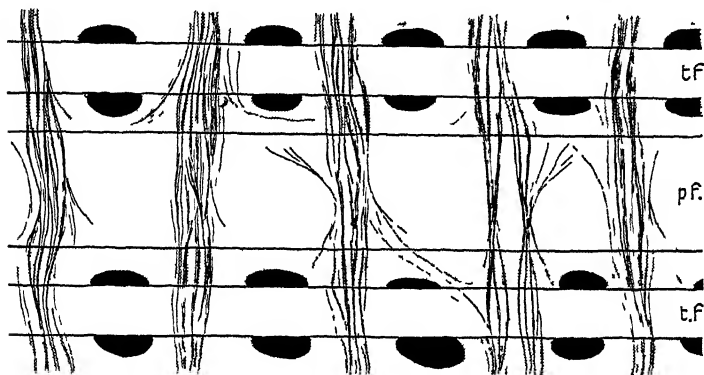
Contraction of the horizontal muscles increases the folding of the demibranch, and, when strong, the grooves may be entirely hidden and the apices only of the plicae exposed. The utilization of the plicae in the sorting of material in *Pinna* has been described in a previous paper (Atkins, 1937, p. 347). The horizontal muscles assist the longitudinal muscles of the axis and free edges of the demibranchs in diminishing the length of the gill.

(d) *Ostreacea*.

Chitinous supporting structure of the principal filaments.—In *Ostrea edulis*, the only member of the *Ostreacea* investigated, it consists of two bars supporting the frontal and lateral surfaces to some little distance beyond the position of the lateral cilia. It is similar to that of the ordinary filaments, but much stouter. The abfrontal surface appears to be unsupported.

Horizontal muscles.—Horizontal muscle-fibres are abundant, but are practically confined to the region of the numerous interfilamentar junctions (Text-fig. 21): they pass across the inner ends of the chitinous bars of the principal

filaments. As noted by Ridewood (1903, p. 218) 'most of the interfilamentar junctions are bands of tissue running horizontally round the inner surface of the plica, but each third or fourth in a vertical series extends across the plica as a horizontal septum'. In the first instance the horizontal muscles run round the inner surface of the plica; in the second there are in addition muscle strands passing directly across the intraplical area to the



TEXT-FIG. 21.

Ostrea edulis. One principal and two adjacent ordinary (transitional) filaments in frontal view to show horizontal muscles. *pf*, principal filament; *tf*, transitional filament. Water pores shown in black; subfilamentar tissue stippled. Unstained, examined in glycerine. $\times 112$.

adjacent principal filament, as Yonge figured (1926, fig. 6, p. 303).

Contraction of the horizontal muscles diminishes the length of the demibranchs and increases the folding.

THE INTRAFILAMENTAR SEPTUM.

The horizontal muscle-fibres so far described as crossing the principal filaments are distinct from the intrafilamentar 'septum' (*tm*, figs. 8, 9, Pl. 15), which is considerably more abfrontal in position, and when present is found in both principal and ordinary filaments. This septum was considered, by

Ridewood (1903) among others, to be composed of chitin; this is almost certainly incorrect (Kellogg, 1892; Dakin, 1909 *a*). Drew (1906) and Pelseneer (1906, p. 231) thought it to be connective tissue, but it is most probably composed of muscle-fibres or cells (Lucas, 1931; Atkins, 1931). In entire living principal filaments, for instance of *Chlamys opercularis*, viewed from the abfrontal surface, the intrafilamentar 'septum' has the appearance of numerous very closely arranged fine fibres, distinct from those relatively coarse muscle-fibres which are responsible for movements of the lateral extensions of these filaments (see p. 231). Ridewood took the septum to be a continuous membrane in many bivalves, and (1903, pp. 170-2) gave lists of those in which the septum is said to be 'present', 'present at intervals', and 'absent'. As regards the first term he stated: 'The word "present" means that the septum is so commonly visible in the sections, that its occasional absence may be regarded as due to the rupture of the membrane in the act of cutting.' It is possible that the muscle-fibres are connected by a thin membrane, though such was not seen. Drew's experience in injecting ordinary filaments of *Pecten tenuicostatus* is against there being such a membrane, for he found that the whole of the cavity of the filament was filled as far as the injection extended. He concluded that this indicated that the apparent partition is not functional as a division between vessels. Its function is probably as Drew suggested, to act as a brace to keep the filament from swelling into a cylinder with the pressure of blood and so obstructing the flow of water between the filaments.

The muscle-fibres appear to be inserted on the darkly-staining chitin of the filaments, which in some bivalves, for instance in *Arca tetragona* and *Spondylus gaederopus*, is thickened beneath the insertion. It may be that their ends are embedded in the chitin as Lucas (1931, p. 168) thought to be the case in *Mytilus edulis* and *Modiolus modiolus*.

SUMMARY.

In the gill axes of the Microciliobranchia the most important muscles are longitudinal and transverse.

The longitudinal muscles are: (a) those extending from one extremity of the gill axis to the other, inserted on the shell anteriorly, and (b) those in the free posterior portion of the axis, inserted on the shell where the axis becomes attached. Together these muscles act as branchial retractors. Withdrawal of the gills prevents (a) their being caught and crushed by the edges of the shell when the valves are suddenly closed, and (b) excessive fouling with sudden intake of muddy or noxious water.

The transverse muscles below the chitinous structure arching the axial food groove serve to draw the demibranchs of a gill together, while those above the arch serve to separate them. Such swaying movements of the demibranchs serve to rid them of unwanted material.

In the demibranchs are:—(1) muscles of the free edges. These include (a) muscles responsible for movements of the walls of the food grooves, and (b) longitudinal muscles, which effect antero-posterior contraction and assist the longitudinal muscles of the axis in retraction of the gills; (2) vertical muscles of the demibranchs, found chiefly in the Pterioacea, and responsible for dorso-ventral contraction of the demibranchs; (3) muscles of the interlamellar junctions serving to draw the two lamellae of a demibranch together, expelling the contained water; (4) horizontal muscles of the lamellae, present in forms with plicate and heterorhabdic gills and effecting by their action changes in shape of the frontal surface of the principal filaments and movements of the plicae important in connexion with the ciliary sorting mechanism; their contraction increases the folding of the lamellae and decreases the length of the gill: and (5) fine muscle-fibres forming the intrafilamentar 'septum'.

NOTE. Part I, 1936, p. 200, line 10: (on *Nuculana minuta*) the small rotating masses appear to be thrown occasionally from one leaflet to the next, and very slowly to collect at a point on the gill near the posterior edge of the foot.' As this has been taken (Yonge, 1939, Protobranchiate Mollusca, etc., Phil. Trans. Roy. Soc. Lond., B, 230, p. 104) to be a statement that material 'tends to be thrown forward from filament to filament by muscular and not ciliary action and so finally conveyed to the rejection tracts on the posterior margin of the foot', I should perhaps make it clear that the rotating masses are thrown forward by the action of the cilia which are rotating them: no mention was made of rejection tracts on the foot; the cilia on it beat dorsally.

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EXPLANATION OF PLATE 15.

ABBREVIATIONS USED IN THE PLATE.

cj, ciliary junction; *dch*, darkly-staining chitin; *hm*, horizontal muscles; *ic*, interlocking cilia; *ils*, interlamellar septum; *lc*, lateral cilia or position of lateral cilia; *m*, muscle?; *mgl*, mucous gland; *nu*, nucleus; *pc*, pigment cells; *pch*, pale-staining chitin; *sp*, spur bearing interlocking cilia; *spm*, spur muscles; *tm*, transverse muscle-fibre of intra-filamentar septum; *vertm*, vertical muscle; *vertm*¹, vertical muscle in lateral position; *vertm*², vertical muscle which in lower or ventral part of demibranch becomes abfrontal in position; *vertn*, vertical nerve; *vertn*?, vertical nerve?

Figs. 1 to 11 are transverse sections of principal filaments of various members of the Microciliobranchia to show the disposition of the muscles. Figs. 3 to 9 are from museum material in which the preservation is imperfect, especially of the frontal epithelium, and this has had to be largely reconstructed.

Fig. 1.—*Pteria hirundo* (adult). Two principal filaments with connecting septum and adjacent ordinary filaments from near ventral margin of demibranch. One of the principal filaments is shown in ciliary junction with adjacent ordinary filaments. In this figure and in figs. 2, 4–7 markings in the chitin supporting the wings or lateral extensions of the filaments are evident. Bouin-Duboscq's fixative: iron haematoxylin and acid fuchsin. $\times 337\frac{1}{2}$.

Fig. 2.—*Pteria hirundo* (adult). Principal and attached ordinary filament from dorsal region of descending lamella: organic in addition to ciliary junction as shown here is unusual. Section slightly oblique. Bouin-Duboscq's fixative: Mallory's triple stain. $\times 337\frac{1}{2}$.

Fig. 3.—*Pinctada vulgaris*. From dorsal region, descending lamella and passing through the spurs (*sp*). Alcohol preservation: Mallory's triple stain. $\times 430$.

Fig. 4.—*Pinctada margaritifera*. From descending lamella: section passing through spurs (*sp*) and showing ordinary filaments attached by compound ciliary and organic union. Alcohol fixation: iron haematoxylin and acid fuchsin. $\times 250$.

Fig. 5.—*Pinctada margaritifera*. From ascending lamella; not passing through spurs. Alcohol preservation: Mallory's triple stain. $\times 250$.

Fig. 6.—*Pinctada margaritifera*. Same principal filament as shown in fig. 5, but passing through spurs (*sp*) and showing adjacent ordinary filaments attached to it by compound ciliary and organic union. Figs. 5 and 6 are of sections about $130\ \mu$ apart. Alcohol preservation: Mallory's triple stain. $\times 250$.

Fig. 7.—*Vulsella* sp. Section passing through spurs (*sp*). Alcohol preservation: iron haematoxylin and acid fuchsin. $\times 430$.

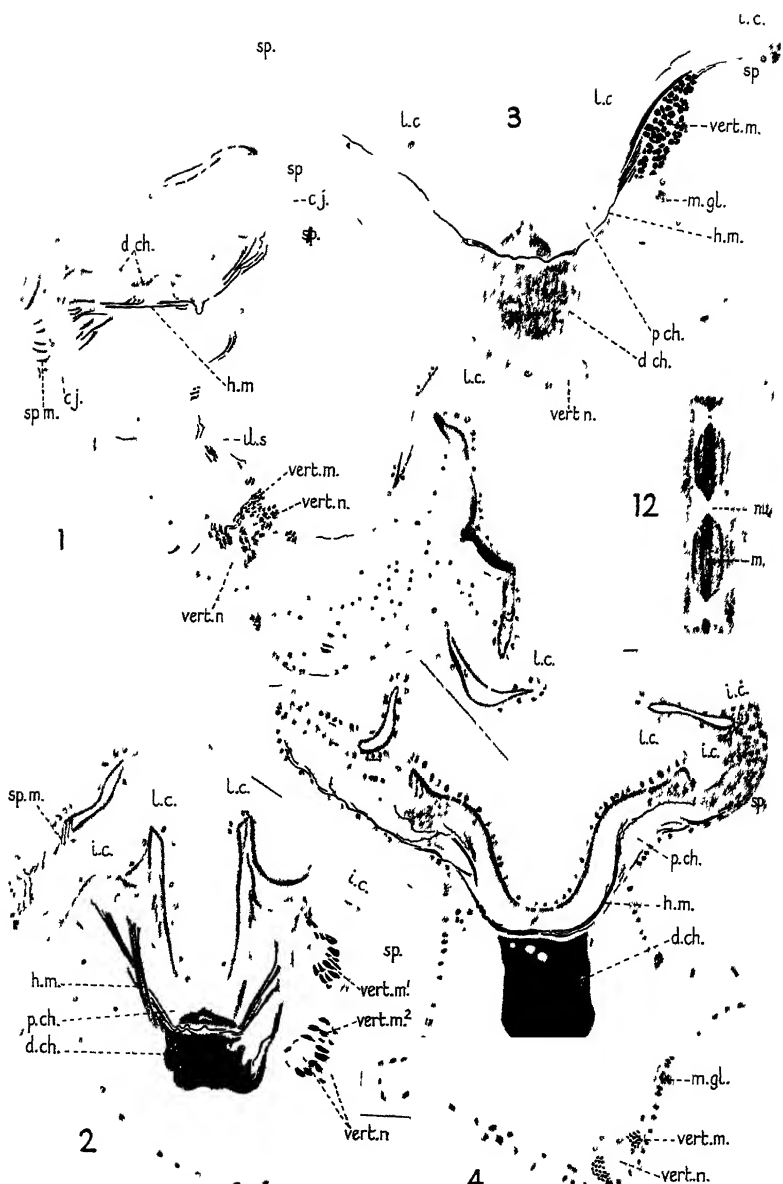
Fig. 8.—*Amussium pleuronectes*. Section through region of principal filament where no horizontal muscles pass through the chitinous tube. Cilia not shown as preservation imperfect. Alcohol preservation: Mallory's triple stain. $\times 430$.

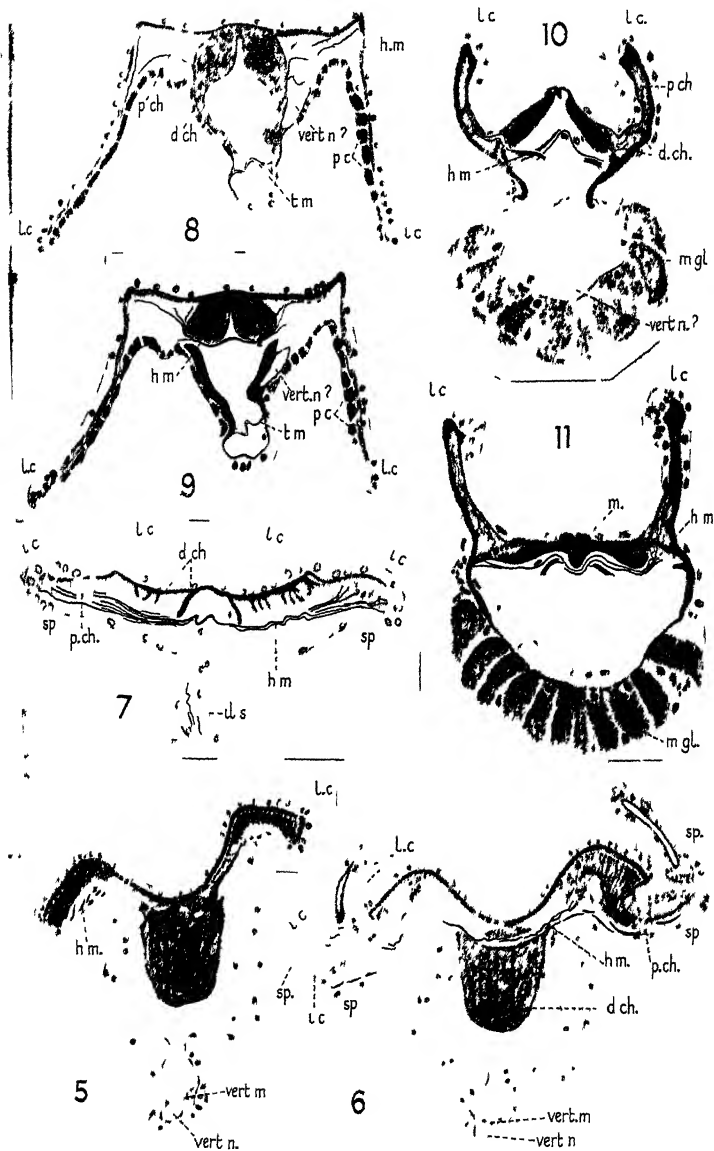
Fig. 9.—*Amussium pleuronectes*. Same principal filament as shown in fig. 8, but sectioned in region where horizontal muscles pass through the chitinous tube: figs. 8 and 9 are of sections about $6\ \mu$ apart. Alcohol preservation: Mallory's triple stain. $\times 430$.

Fig. 10.—*Lima hians*. Showing ridge arising from bottom of frontal groove of principal filament. Bouin-Duboscq's fixative: Mallory's triple stain. $\times 430$.

Fig. 11.—*Lima hians*. Frontal groove of principal filament with almost flat bottom. Bouin-Duboscq's fixative: Mallory's triple stain. $\times 430$.

Fig. 12.—*Lima hians*. Sketch of chitinous skeleton of principal filament as it appears in longitudinal section just below and parallel with the frontal surface. Bouin-Duboscq's fixative: iron haematoxylin and acid fuchsin. $\times 430$.





Tegumental Glands in the Cirripedia Thoracica.¹

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With 11 Text-figures.

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1. INTRODUCTION.

WORKING on Decapod Crustacea, in particular on *Homarus vulgaris*, Yonge (1932) showed that the exo-skeletal integument consists of two portions, a thin superficial cuticle, which is hard and relatively impermeable, containing adsorbed lipin, and underlying this a thick, relatively soft, freely permeable chitin. These two layers are structurally and chemically distinct and have different origins, the cuticle being formed not by the chitinogenous epithelium but by numerous tegumental glands situated beneath the epidermal epithelium. The presence of similar glands with intracellular ducts was also demonstrated in other groups of the Crustacea.

The fundamental significance of the cuticle as a protective and insulating layer was shown not to be incompatible with

¹ Owing to Dr. H. J. Thomas's absence on active service this paper, part of his thesis for the degree of Doctor of Philosophy, has been prepared for publication by Mr. R. Bassindale.

accessory functions such as the binding of the eggs to the pleopods of the female (Yonge, 1938), the attachment of sand grains to the setae in the statocysts (Lang and Yonge, 1935), and the formation of threads constituting the nest of certain Amphipoda, e.g. *Ampelisca* (Yonge, 1932).

The present investigation of the tegumental glands of the Cirripedia was undertaken in the light of the above work. The greater part of it was carried out at the University of Bristol, experiments on living material being conducted at the Plymouth laboratory of the Marine Biological Association during the spring of 1938. Thanks are due to Professor C. M. Yonge at whose suggestion and under whose supervision the investigation was conducted, and also to Mr. R. Bassindale, M.Sc., for valuable advice.

2. MATERIAL AND METHODS.

The following species were obtained fixed in formalin and also in either Bouin's fluid or corrosive sublimate or both: *Lepashilli* Leach from Tortugas; *Lepas anatifera* Leach (also in Zenker) *Scalpellum scalpellum* Leach, *Balanus perforatus* Brugière *Balanus balanoides* Leach, and *Alcippe lampas* Hancock, all from Plymouth. In addition specimens of *Lithotrya valentiana* Gray in 90 per cent. alcohol from the Great Barrier Reef were available.

Fixation of the tegumental glands was good in specimens treated with 10 per cent. formalin, Bouin, Zenker or corrosive sublimate, the last being probably the best. The specimens of *Lithotrya valentiana* were of little histological value, but permitted the determination of the distribution of tegumental glands and of the distribution and staining reactions of their secretions.

Microtome sections were cut from 4 to 8 μ thick. Good results for general purposes were obtained by staining in Delafield's haematoxylin with eosin as a counterstain, but for finer details Heidenhain's haematoxylin counterstained with Biebrich scarlet was most suitable. The demarcation between chitin and other secretions was best displayed by Mallory's triple stain.

Hand sections 10 to 20 μ thick were cut from the integument

of all regions of large *Lepas anatifera* (preserved in 10 per cent. formalin) and subjected to stains and microchemical tests. The iso-electric points of both layers of the integument were determined following the technique used by Yonge (1932) and in addition small pieces of the integument were subjected to Campbell's modification of Van Wisseligh's test for chitin (Campbell, 1929).

Owing to the thinness of the cuticle on the chitin outside the mantle cavity it was not possible to observe its reaction with reagents acting on hand sections. However, by decalcifying the calcareous plates from large *Lepas anatifera* the thin superficial cuticular covering was obtained. This proved suitable for examination.

3. NATURE OF THE INTEGUMENT.

In the pedunculate Thoracica, e.g. *Lepas*, *Scalpellum*, *Lithotrya*, the mantle and the enclosed body forms the capitulum and there is a long stalk, the peduncle, which attaches the capitulum to the substratum. In the sessile Thoracica, e.g. *Balanus*, the peduncle is absent and the calcareous plates of the capitulum articulate to form a solid wall.

The chitinous exo-skeleton may be divided into two regions, that covering the outer surface of the capitulum and peduncle, which may include numerous calcareous plates, and that lining the mantle cavity and covering the body proper. The latter or inner integument is thin and is shed regularly at the moult. The former or outer integument is thick and laminated, due to the fact that, except on the peduncle of *Lithotrya*, it is not shed at the moult, thus recalling similar incomplete moults of the Conchostraca and some Cladocera.

From the examination of microscopic and hand sections and by means of the tests employed by Yonge (1932) and Campbell (1929) it was established (see Tables I and II below) that in all species examined the integument consisted of a thick layer of chitin underlying a much thinner layer of cuticle—both layers being continuous over the whole surface of the body. In mature *Lepas hilli* the chitin varied in thickness from $250\ \mu$ on the peduncle to $3\ \mu$ in the hind gut, and the cuticle, with an average

thickness of some $1.5\ \mu$, varied from $10\ \mu$ on the fused 2nd maxillae (labium or lower lip) to $0.4\ \mu$, in the hind gut. Ducts passing through the integument were present in all species examined and similar ducts run through the calcareous valves of the capitulum of some species.

4. TEGUMENTAL GLANDS.

There are three types of tegumental glands: (a) those scattered over the outer integument, (b) the cement glands, and (c) those usually termed 'salivary' glands situated on or near the 2nd maxillae. The last two types are well known. The cement glands are concerned with the attachment of the animal to the substratum and not, as are the other two types, with the secretion of the body cuticle.

(a) The Glands of the Peduncle and outer Surface of the Capitulum in four Pedunculate Species.

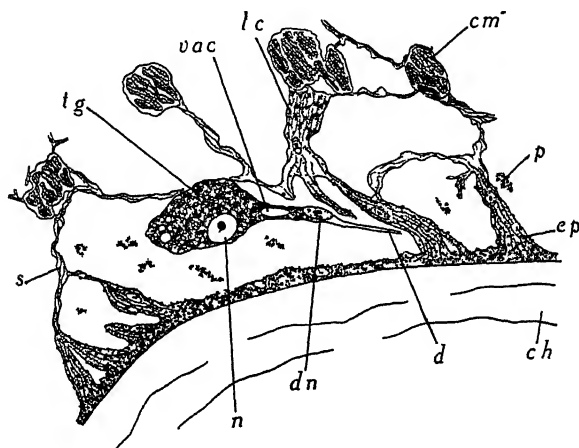
The organs in the body of the Cirripedia are held in a spongy lacunar tissue which gives way at the surface to a regular epithelium consisting of a single layer of striated conical cells occasionally interrupted by cubical cells. Immediately below this epithelium lie the individual cells of the tegumental glands, and although these glands have not been previously described the ducts passing through the chitin of the peduncle were mentioned by Darwin (1851) and others (see discussion).

(i) *Lepas hilli*.

In *Lepas hilli* there are numerous gland-cells beneath the epithelium underlying the integument of the peduncle (Text-fig. 1, *tg*). Each cell is large ($40\ \mu$ long) and roughly oval in section, although somewhat irregular due to the connective tissue strands which hold it in position. The actively secreting cell has a large central nucleus. After fixation in Bouin's fluid or 10 per cent. formalin this shows a few large granules and a prominent nucleolus. The cytoplasm contains numerous rounded vacuoles (*vac*) which in the region opposite the origin of the duct may attain considerable dimensions. Numerous small granules, presumably secretion, are also present; these are generally

rounded and have affinity for both cytoplasmic and nuclear stains, particularly for the former. The cytoplasm of the inactive gland-cell is practically homogeneous, without vacuoles or granules, being lightly coloured by nuclear stains and unaffected by cytoplasmic stains.

At one end of its long axis the gland-cell merges into a duct



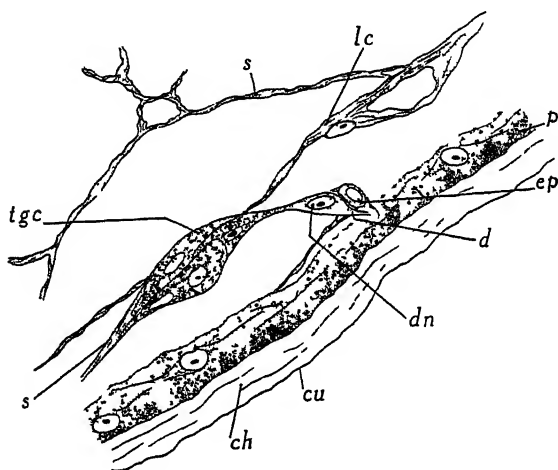
TEXT-FIG. 1.

Lepas hilli. Longitudinal section of stalk showing unicellular tegumental gland. $\times 700$. *ch*, chitin; *cm*, circular muscle; *d*, intracellular duct; *dn*, duct nucleus; *ep*, epithelium; *lc*, lacunar tissue; *n*, nucleus; *p*, pigment; *s*, branching connective tissue strands; *tg*, tegumental gland; *vac*, vacuole.

of which the lumen may project considerably into the cytoplasm of the gland-cell, or in some cases a large vacuole may be present (Text-fig. 1, *vac*). This short funnel-shaped portion of the duct formed by the gland-cell is wide and somewhat irregular; it leads immediately to a separately formed intracellular duct (*d*) which is long, narrow, and of uniform bore. At the junction of the duct from the gland-cell and the intracellular duct is situated a large duct nucleus (*dn*). This is typically elongated, flattened, and closely applied to the surface of the duct. It possesses a number of deeply staining granules and has a fairly prominent nucleolus. The duct nucleus is surrounded by a

narrow layer of clear cytoplasm similar to that forming the walls of the duct. This is unbranched and runs for some way through the epithelium, parallel to the integument, before turning outwards as a much coiled tube leading through the epithelium and integument, and opening by a pore to the exterior.

In addition to these large unicellular glands associated with



TEXT-FIG. 2.

Lepas hilli. Longitudinal section of the capitulum showing compound tegumental gland from the chitinous region below the adductor muscle. $\times 700$. *cu*, cuticle; *tgc*, compound tegumental gland. Other lettering as before.

the stalk region there are compound tegumental glands underlying the epithelium on the outside of the capitulum. These consist of groups of five or six small gland-cells (15μ long) opening into a common intracellular duct leading to the exterior (Text-fig. 2, *tgc*). Histologically, these cells are similar to the unicellular glands described above. The nucleus, however, is longer in proportion to the size of the cell and a prominent nucleolus is not so characteristic a feature.

Depending on the position, the glands may be compact or diffuse. In regions where the epithelium consists largely of

cubical cells (e.g. below the adductor muscle) the gland is compact (Text-fig. 2). In this case collecting ducts are short and fine, the condition being similar in many ways to that found in Decapoda. If, however, a copious lacunar tissue is present, the tegumental gland is diffuse, with long, wide collecting ducts (much as in *Scalpellum*, Text-fig. 6), a condition recalling that in the cement glands.

As in the case of the unicellular glands, each cell narrows off at one end, giving rise to a short, wide, irregular duct, formed by the gland itself. These collecting ducts join together and are continuous at their outer end with the separately formed main intracellular duct, which bears at its upper end a typical duct nucleus. The duct itself is of uniform bore, fine and unbranched, being similar to that of the unicellular glands, though usually of greater length.

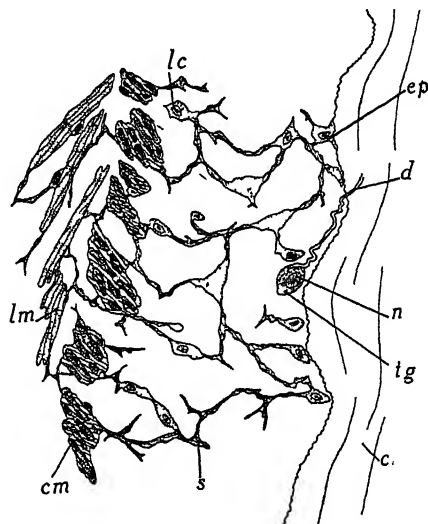
In certain cases, the duct originating from a compound tegumental gland bears in the typical position of the duct nucleus a cell of glandular appearance (Text-fig. 2, *dn*). The nucleus of this cell is surrounded by a granular cytoplasm which, in comparison with the cytoplasm of the typical tegumental gland, is markedly reduced and possessed of considerably fewer granules. The intracellular duct passes through the protoplasm. It is quite short and the condition suggests a developmental stage. Presumably the compound gland originates from a small secretory cell with a short duct leading directly to the exterior and bearing no duct nucleus, similar to the gland-cells of the stalk of *Lepas anatifera* (Text-fig. 3, *tg*). As further secretory cells become differentiated, the initial cell gradually loses its glandular function and, with loss of its granules and the reduction of its cytoplasm, becomes specialized for the formation of a duct of increasing length. In this way the nucleus of the initial gland-cell becomes a duct nucleus, associated with the long intracellular duct leading from the compound tegumental glands.

(ii) *Lepas anatifera*.

The tegumental glands underlying the integument of the stalk of *Lepas anatifera* are of the simplest type examined.

Numerous small unicellular glands, about $10\ \mu$ in diameter, occur well within the epithelial layer (Text-fig. 3, *tg*). The gland-cell is somewhat larger than the ordinary epithelial cells, and leads by a short and little coiled duct directly to the exterior. There is no duct nucleus.

Underlying the integument of the outside of the capitulum



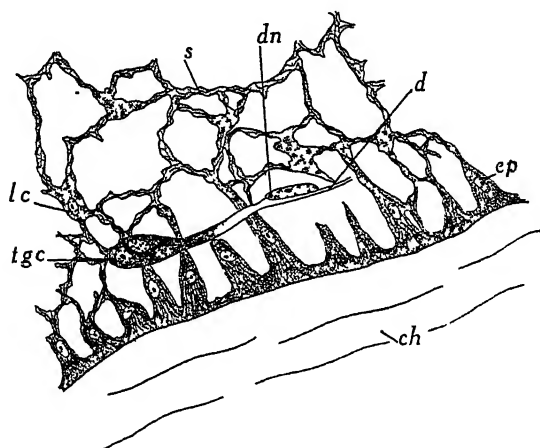
TEXT-FIG. 3.

Lepas anatifera. Longitudinal section of stalk showing unicellular tegumental gland. $\times 700$. *lm*, longitudinal muscle. Other lettering as before.

are compound tegumental glands, the individual cells of which are about $15\ \mu$ in length (Text-fig. 4 *tgc*). They are situated inside the epithelial layer and histologically are similar to those of the capitulum of *Lepas hilli*. Leading from the gland is a long, coiled duct (*d*) bearing a typical duct nucleus (*dn*). The majority of these ducts pass through the integument in a typical manner. They are of uniform bore and follow a much coiled course to the exterior, where they open by a fine pore. In the region of the integument between the calcareous plates of the capitulum certain of the ducts, which in this region are of

particularly fine bore, bear a rounded swelling before opening to the exterior. These are described and figured by Gruvel (1905) under the name of 'organes vésiculeux'.

In one specimen the integument in the upper part of the stalk showed signs of damage (Text-fig. 5, *chd*). Large amounts of



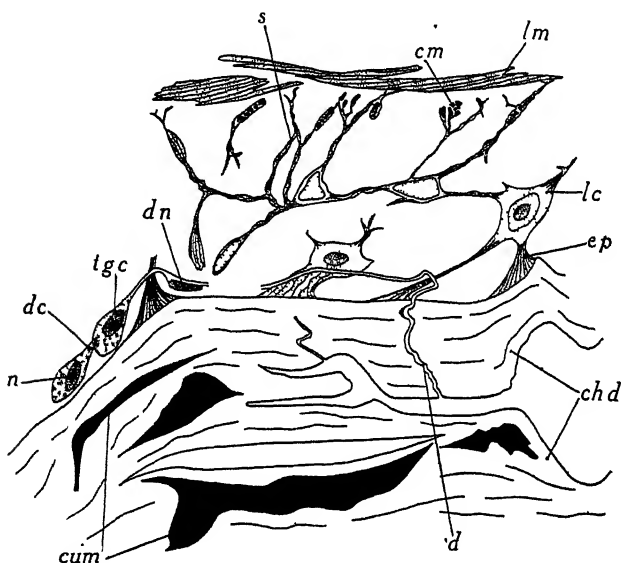
TEXT-FIG. 4.

Lepas anatifera. Longitudinal section of capitulum showing compound tegumental gland. $\times 700$. Lettering as before.

cuticular material were observed in the tissues within the chitin of the damaged area (*cum*). Underlying such regions are large, diffuse, compound tegumental glands. The component cells of these are of greater size (17μ in diameter) than the normal unicellular gland of the stalk region, and are characterized by a dense nucleus with a very pronounced nucleolus. The gland-cells are flask-shaped and lie within the epithelium immediately below the chitin. At one end of its longitudinal axis the cell gives rise to a long, somewhat irregular and tapering collecting duct (*dc*). A number of these ducts join together conveying the secretion from the plate-like area of gland-cells to the main intracellular duct which is exceptionally long, and bears at its upper end a typical duct nucleus (*dn*).

(iii) *Scalpellum scalpellum*.

In this species the whole of the outer integument, including that of the stalk, possesses calcified areas. Associated with this integument are compound tegumental glands of a type similar to those underlying the calcified capitulum of *Lepas hilli*

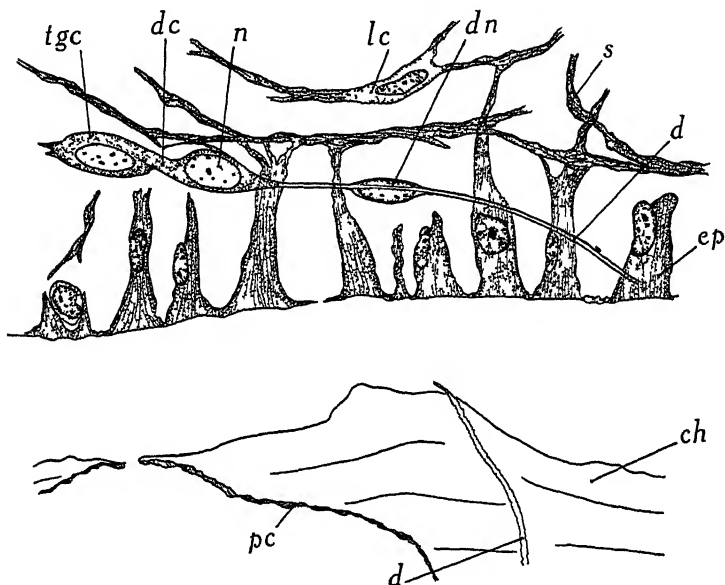


TEXT-FIG. 5.

Lepas anatifera. Longitudinal section of the stalk showing compound tegumental glands in the region of damaged integument. $\times 700$. *chd*, damaged chitin; *cum*, mass of cuticular substance; *dc*, collecting duct of gland-cell. Other lettering as before.

and *Lepas anatifera*. The glands are diffuse and the component cells large (30μ in diameter). The nucleus (Text-fig. 6 *n*) is exceptionally large and after fixation in Bouin's fluid a nucleolus is not apparent. The gland-cells are joined together by wide irregular collecting ducts which pass to a long, somewhat narrow intracellular duct. This bears a typical duct nucleus (*dn*), is slightly coiled, and leads through the epithelium and integument.

Passing through the integument the ducts follow the typical much coiled course. In the region of the capitulum they traverse the calcareous plates. In the stalk region on the other hand the ducts run around the scales, but their openings are uniformly distributed at the surface. The external opening is a



TEXT-FIG. 6.

Scalpellum scalpellum. Longitudinal section of stalk region showing compound tegumental glands. $\times 700$. *dc*, collecting duct of gland-cell; *pc*, cuticle lining cavity of calcareous scale. Other lettering as before.

pore of the same diameter as the duct and in general lies flush with the surface of the cuticle. Sometimes, however, it is mounted at the end of a short, stout, chitinous papilla.

(iv) *Lithotrya valentiana*.

Ducts from the tegumental glands are readily detected in the integument on account of the characteristic staining of the contained secretion, which differs markedly from the chitin.

Thus, although the material available was not suitable for histological study, the distribution of tegumental glands was readily determined.

Ducts with the typical characteristics are common in the whole of the chitin of the stalk, and of the chitinous parts on the outside of the capitulum. As in the case of the other genera examined no ducts were found in the chitin lining the mantle cavity or covering the body proper.

Beneath the epithelium large glandular masses were observed apparently very similar to the unicellular glands of the stalk of *Lepas hilli*.

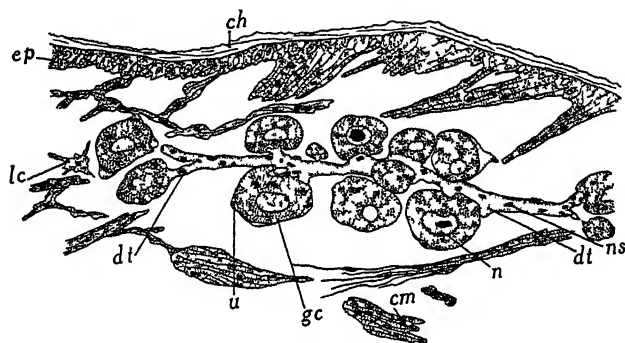
(b) Cement Glands.

In addition to the tegumental glands above described there are, in the peduncle of pedunculate thoracic cirripedes, well developed glands which secrete a substance which attaches the animal to the substratum. Similar glands are found in the basis of operculate thoracic cirripedes. These cement glands are well known, and their gross anatomy has been described by both Darwin (1851) and Gruvel (1905). The glands normally consist of paired masses of cells whose secretion is collected by two main ducts which may or may not unite before opening at the base of the peduncle in the Pedunculata, or in the centre of the basis in the Operculata. Slight variations occur in different species, the most notable being in *Lithotrya* where, from a swelling in the duct, fine canals discharge cement through a calcareous attachment plate at the base of the peduncle.

In *Lepas anatifera*, which is typical of the Pedunculata, each gland-cell is roughly spherical and between 30 and 40 μ in diameter (Text-fig. 7). The cytoplasm of an actively secreting cell is finely granular and without vacuoles. The nucleus has a large nucleolus, and fine granules are sometimes present in addition. Certain variations in the appearance of the cells indicate that they undergo a secretory cycle identical with that described by Krüger (1923) for the cement glands of *Scalpellum scalpellum* and *Scalpellum stromii*.

The branched ducts, which project slightly into the gland-cells, are of uniform bore, and consist of a syncytium with

evenly distributed granular nuclei. The main duct is similar except that the nuclei are less numerous and the cells consequently more flattened. Small flattened cells, with nuclei and cytoplasm identical with those of the collecting ducts, are frequently seen adpressed to the gland-cells (Text-fig. 7, *u*). These are presumably products of the proliferation of cells which



TEXT-FIG. 7.

Lepas anatifera. Longitudinal section of stalk showing part of the cement gland. $\times 300$. *dt*, tributary duct from the gland-cell; *gc*, cement gland cell; *ns*, nuclei of duct syncytium; *u*, undifferentiated cells. Other lettering as before.

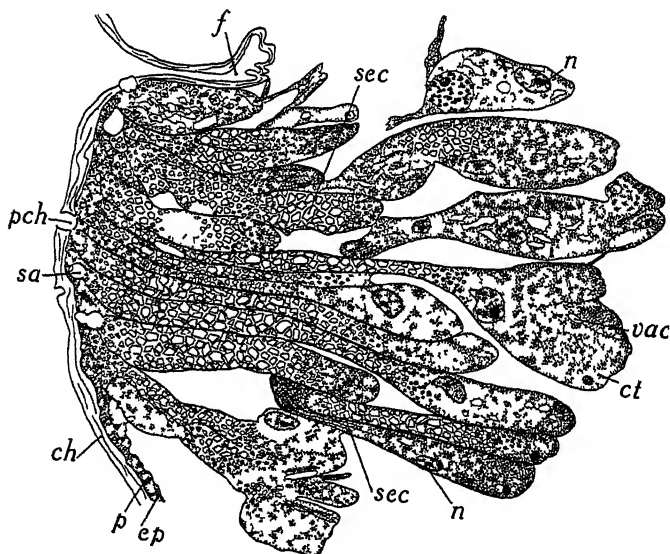
has given rise to the gland mass and the ducts. Probably they are continuous with the cells of the collecting duct, and with them form an envelope around the gland-cells.

(c) 'Salivary' Glands.

Large masses of unicellular glands open on the fused second maxillae or labium of Cirripedia Thoracica, and similar glands open near the base of the first, second, or third thoracic appendages. These have been termed 'salivary' glands although attention has been drawn to their unsuitable position, particularly of those at the base of the appendages. They have been figured by Gruvel (1905), and secretory material is a very prominent feature in the very large cells. The glands of *Lepas hilli* (Text-fig. 8) are typical and hand sections of the integu-

ment overlying them shows that the chitin is covered by a special thick cuticle which is under tension and often tears away in paraffin sections (as in the Decapoda; Yonge 1932).

Gruvel (1905) ascribed to the 'salivary' glands the production of a 'bol alimentaire' which cements together food particles scraped off the cirri and which is swallowed with the contained



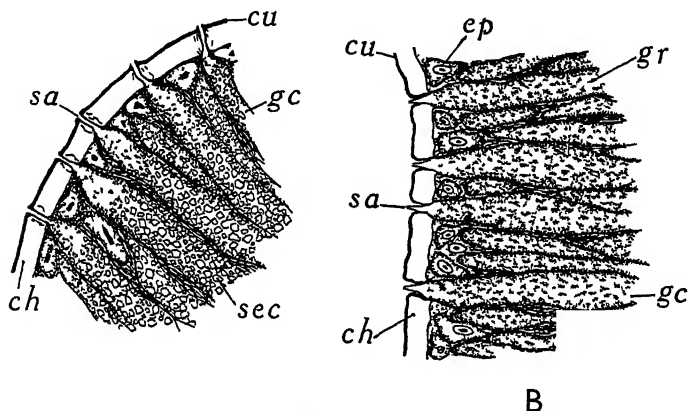
TEXT-FIG. 8.

Lepas hilli. Longitudinal section of the capitulum showing the 'salivary' gland. $\times 300$. *ct*, cytoplasm; *f*, fold of chitin; *pch*, chitin pit; *sa*, secretory pore of cell; *sec*, large secretory agglomerations. Other lettering as before.

food particles. Broch (1919) pointed out their unsuitable position, since even the labial gland has many openings on the lateral, outer face of the second maxillae, and the other glands are more remote from the mouth. He suggested that the glands serve to poison the prey.

Since living pedunculate material is rare, these glands were studied in the large operculate barnacle *Balanus perforatus*. Here, the second pair of glands at the cirrus base

(and underlying the suboesophageal ganglion) are identical in appearance with the glands of *Lepas hilli* in that the cells open flush with the surface by ducts of uniform bore (Text-fig. 9 A) and the secretion is in the form of coarse agglomerations. The labial glands of *Balanus perforatus*, however, differ in that the secretion is more often finely granular (after the same fixative), although coarse granules may sometimes be present



TEXT-FIG. 9.

Balanus perforatus. Semi-diagrammatic representation showing the difference between (A) the glands of the suboesophageal ganglion and (B) the glands of the second maxillae. \times approx. 300. *gc*, 'salivary' gland-cell; *gr*, secretion forming fine granules; *sa*, secretory pore of gland-cell. Other lettering as before.

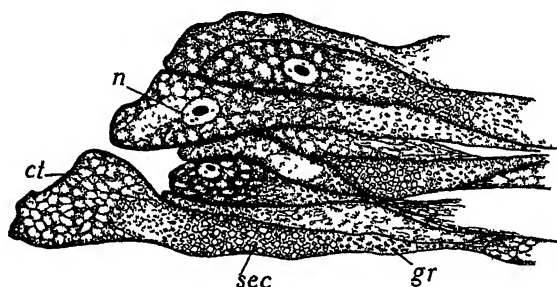
in the inner half of the cell (Text-fig. 10). In addition the secretory pore is in the form of a conical nozzle situated in a pit in the chitin (Text-fig. 9 B).

Balanus perforatus is easy to keep alive in circulating sea-water and individuals of similar size were chosen for experiment. Moults occurred about every fifth day, and specimens were fixed at varying periods after the moult. In addition individuals were placed for varying periods in thick suspension of carmine particles or blood corpuscles and then fixed.

In a carmine suspension the barnacle was observed to make regular sweeps with its feeding cirri, and then to discard from the mantle cavity a carmine-coloured mass which consisted of

carmine particles bound in a tangle of threads of a hyaline non-sticky substance. This substance was found to be unaffected by digestive juices from the alimentary canal, nor was it ever discovered in the alimentary canal. It seems, therefore, that the 'bol alimentaire' of Gruvel is formed but not swallowed, and that its formation is associated with the rejection of unwanted particles.

Specimens subjected to a thick suspension of carmine particles



TEXT-FIG. 10.

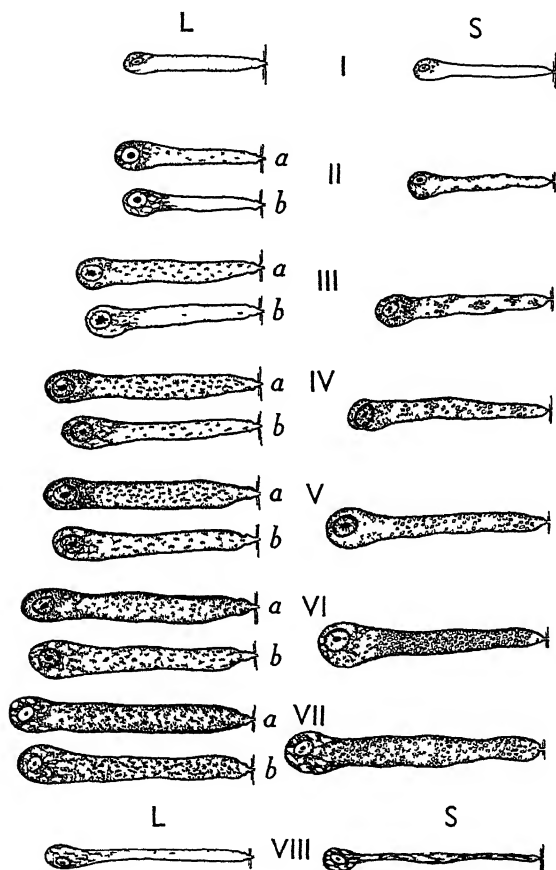
Balanus perforatus. Longitudinal section showing salivary gland-cells. $\times 300$. *gr*, secretion in form of fine granules. Other lettering as before.

were capable of rejecting two masses of aggregated particles, but after this the cirri became clogged and collecting soon ceased. Furthermore, such animals could not perform the function of rejection even two days after such treatment. It seemed that the source of the entangling material was exhausted, although sections often showed the presence of considerable amounts of secretory material within the gland-cells of the 'salivary' glands of the maxillae. Animals fed on blood corpuscles showed no signs of secretion from either set of glands.

In order to make a chemical examination of the entangling material finely ground silver sand was allowed to fall across the cirri of a feeding barnacle, and the rejected mass of entangled sand particles was subjected to the tests described later.

The glands were obviously not concerned exclusively with the rejection of material, and their condition was examined in sections of animals fixed at varying periods after the moult. The

activity of both labial and suboesophageal glands showed a marked correlation with moulting. The secretory cycle is



TEXT-FIG. 11.

Balanus perforatus. Diagrammatic representation of the secretory stages of the 'salivary' glands. L. The glands of the second maxillae, *a*, before secretion of rejectory material; *b*, after secretion of rejectory material. S. The glands in the region of the suboesophageal ganglion. \times approx. 400.

illustrated in Text-fig. 11 for both suboesophageal (s) and labial (L) glands. The series *a* and *b* of the labial glands indicate

the difference between animals fed normally (*a*) and animals subjected to carmine suspensions and so exercising the rejection mechanism (*b*).

At the moult, or just after it, cells of both glands are devoid of secretions (Text-fig. 11, viii). Subsequently in the suboesophageal glands (Text-fig. 11 s, i-vii), and in the labial glands of animals feeding normally (Text-fig. 11 L, i-vii, *a*), the cells gradually accumulated secretory material, becoming large and heavily charged immediately prior to the moult. After the moult the cells are shrunken and possess a small nucleus with a few granules. The cytoplasm is homogeneous and vacuolated. Soon the cells enlarge, but appear empty except for the granular cytoplasm surrounding the now nucleolate nucleus at the base. The cell continues to grow, and the cytoplasm contains more granules and increases in amount. Prior to the moult the granules of secretion accumulate in the distal two-thirds of the cell and appear as large aggregations after fixation. About this time the cytoplasm at the base becomes vacuolated, and it may be the enlargement of these vacuoles which discharges the secretion. The secretory cycle of the individual cell is identical with that of the cement gland cells of *Lepas* (see above) and of *Scalpellum* (Krüger, 1923).

In an animal fed on carmine particles the glands on the labium show slight modifications from the above cycle. Limited numbers of secretory granules appear near the cell opening, and the cytoplasm develops vacuoles at an early stage. At this period all the available secretion may be discharged during particle rejection (Text-fig. 11 L, ii, *b* and iii, *b*). Later, however, not all the secretion is discharged, and there is a reserve accumulation (Text-fig. 11 L, iv-vii, *b*) which is not discharged during rejection but is preserved for the moult.

5. SECRETIONS OF THE GLANDS.

The integument in Decapod and other Crustacea consists of a thick layer of chitin secreted by the epithederal cells, and of a thin layer of cuticle secreted by special tegumental glands. In the above account the cirripede integument is shown to consist

of similar layers and three types of glands which may correspond to tegumental glands are present.

Tests were applied to identify the chemical nature of the cirripede integument, and Tables I and II summarize the results.

TABLE I.

Reactions of the Chitin and Cuticle of the External Surface, Cuticle of the Mantle Cavity and Cement of *Lepas anatifera*, and of the Rejectory Secretion of the 'Salivary' Glands of *Balanus perforatus*.

	<i>Chitin.</i>	<i>Cuticle of external surface.</i>	<i>Cuticle of mantle cavity.</i>	<i>Cement.</i>	<i>Rejectory secretion of 'salivary' glands.</i>
1. Iso-electric point.	5.0	3.4	3.5	3.5	3.4
2. Conc. HCl.	Partial soln.	Unchanged	Unchanged	Unchanged	Unchanged
3. Warm saturated KOH solution.	Insoluble	Dissolves	Dissolves	Dissolves	Dissolves
4. Sudan III.	Unstained	Pink	Pink	Pink	Pink
5. Osmic acid	Light brown	Black	Black	Black	Black
6. Millon's reagent	Faintly pink	Deep brick red	Deep brick red	Deep brick red	Deep brick red

On addition of hydrochloric acid there was an immediate contraction and a marked solution of the chitin. Complete solution did not occur even on warming. Pure chitin with warm concentrated acid is converted into the soluble glucosamine hydrochloride. The incomplete solution may be due to the effect of the formalin in which the *Lepas* was fixed and which is known to affect amino groups.

Campbell's test for chitin (Campbell, 1929) gave positive results for all regions of the integument (not for the cement), thus confirming the evidence recorded in Table I. especially the iso-electric point (5.0) which agrees with that found by Yonge (1932) for the chitin of *Homarus*. The properties of the cuticle of the external surface and of the mantle cavity, recorded in Tables I and II, also agree with those of the cuticle in *Homarus* (including the iso-electric point at 3.4-5), also with those of the cement which binds the eggs to the pleopods

TABLE II.

Staining Reactions of the Chitin and of the Secretions of the Tegumental, 'Salivary', and Cement glands of *Lepas hilli*, and of the secretion of the glands of the Second Maxillae of *Balanus perforatus*.

		<i>Secretions of</i>			
	<i>Chitin.</i>	<i>Tegu- mental glands.</i>	<i>'Salivary' glands.</i>	<i>Cement glands.</i>	<i>Glands of the 2nd Maxillae of Balanus.</i>

1. Delafield's haematoxylin with eosin.

	Faint blue				
(a) In cells		Red	Reddish purple	Purple	Purple
(b) In ducts		Reddish purple	..	Reddish purple	..
(c) After liberation		Reddish purple	Reddish purple	Reddish purple	Reddish purple

2. Heidenhain's haematoxylin with Biebrich scarlet.

	Pale brown				
(a) In cells		Dark red	Dark red	Dark red	Dark red
(b) In ducts		Dark red		Dark red	
(c) After liberation		Dark red	Dark red	Dark red	Dark red

3. Mallory's triple stain.

	Blue				
(a) In cells	..	Red	Red	Reddish orange	Red
(b) In ducts	..	Reddish orange	..	Reddish orange	..
(c) After liberation	..	Reddish orange	Reddish orange	Reddish orange	Reddish orange

in the Decapoda (Yonge, 1938), and the secretion which attaches sand grains to the sensory setae in the statocysts (Lang and Yonge, 1935). In addition the properties of both the cement in the Cirripedia, and of the labial secretion which aids in rejection in the Operculata, are identical with those of the cuticle indicating a fundamental similarity between all the glands concerned. The presence of adsorbed lipin, indicated by positive reactions with Sudan III and osmic acid, is of special interest.

The presence of this in the cuticle of the Decapoda was experimentally demonstrated by Yonge (1936) through its effects on permeability.

The integument of the Cirripedia, therefore, consists of a thick layer of chitin and a thin superficial layer of cuticle, and the cement, labial, and suboesophageal glands are all modified tegumental glands. The apparent absence of cuticle on that part of the integument covered by cement is a further indication that the cement glands represent the modified tegumental glands of this region, while the manner in which the cement spreads round the base of the peduncle indicates the possession of low surface tension, as postulated by Yonge (1932, 1938) for the cuticle and the egg-binding cement of the Decapoda. Moreover, the compound glands of the capitulum of *Lepas hilli* are very similar to the uterine glands of *Chirocephalus* which secrete the cuticular outer egg membrane (Mawson and Yonge, 1938).

6. DISCUSSION.

Three types of gland have been described, (1) the newly described glands of the outer surface of the barnacle, (2) the cement glands, (3) the so-called 'salivary' glands. All three secrete material having identical properties and similar to the cuticle of the Decapoda. The presence of three different sets of glands in the cirripedes seems to be associated with their sedentary habit. It has been shown in the Decapoda that glands identical with those which secrete cuticle at the moult produce the same substance for cementing purposes, e.g. for attaching eggs to pleopods or sand grains to statocyst setae (Yonge, 1938; Lang and Yonge, 1935). It may be imagined, therefore, that when the cirripedes became sessile the attachment cement was supplied by the available tegumental glands. These have now become specialized as the cement glands and produce a copious secretion.

After the assumption of a sessile habit the acquisition of a very thick protecting layer would be an advantage.¹ This has

¹ The inability of the animals to obtain refuge, like the Decapoda, during the helpless period before the new integument calcifies, would alone render moulting of the outer integument impracticable. C. M. Y.

been developed in the cirripedes by retaining the old integument at the moult in the parts exposed to the environment and by the secretion of calcareous material to form protecting plates. Such a thickened integument would be unsuitable for the body proper and for the lining of the mantle (which is probably used as a respiratory surface), and moulting proceeds normally in these regions. On the outer surface where abrasions must be repaired and where cracks in the chitin occur due to the enlargement of the peduncle, tegumental glands secreting continuously must be present; but for the region within the mantle cavity glands secreting only at ecdysis would suffice. These conditions are fulfilled by the large number of small glands on the outer surface as described in *Lepas hilli* and other species, and by the so-called 'salivary' glands within the mantle.

Histological evidence indicates that, in the Pedunculata, the cells of the tegumental glands of the outer surface and of the labial and suboesophageal glands regenerate after secretion. Krüger (1923) came to similar conclusions as a result of his study of the secretory cycle in the cement glands of *Scalpellum*. But whereas the component cells of the tegumental glands of the outer surface and of the cement glands are not all in the same phase so that the glands secrete continuously, in the labial and suboesophageal glands the cells are in step and secrete only at the moult. In the Operculata the cells of the labial and suboesophageal glands regenerate after secretion, but those of the cement glands degenerate, new gland-cells developing from the walls of the duct (Gruvel, 1905). But here all the cells of the cement glands are in the same phase, secretion occurring only at the moult. This is certainly correlated with the discontinuous growth of the Operculata (Darwin, 1853), cement being required only at ecdysis.

Although the small and unevenly distributed tegumental glands of the outer surface of the Pedunculata are here recognized for the first time, the ducts have been seen previously. Koehler (1889) described 'canals' through the integument of the stalk of *Pollicipes cornucopia* some of which run to the calcareous scales. At the base of the scale, these ducts bear a swelling from which fine 'canals' run through the scale to the

surface. Gruvel (1905) has figured these 'organes de Koehler' as well as the 'organes vesiculeux' of *Lepas anatifera*, and the canals running through and perpendicular to, the chitinous and calcareous parts of the integument of Cirripedia in general. Gruvel attributes a nervous function to these structures and figures the swellings on the 'canals' in the 'organes de Koehler' and 'organes vesiculeux' as cellular structures. Careful examination of the latter showed that in reality they are non-cellular, being nothing more than swellings on the 'canals' which continue through the integument to the surface.

'Organes de Koehler' are not present in the calcareous scales of the peduncle of *Scalpellum scalpellum*, since these differ from those of *Pollicipes* in being completely encased in the chitin, so that the ducts are able to run within the chitin round the scale and open with a uniform distribution on the surface of the integument.

Careful study of the 'canals' in the available species shows that they are in association with ducts from typical secretory cells. Gruvel has stated that they contain prolongations of nerves, but their contents are invariably homogeneous with staining reactions identical with those of the cuticle. Occasionally the 'canals' are completely empty. They represent in fact the continuation of ducts from the tegumental glands (as in *Homarus* (Yonge, 1932)) and serve to carry the secretion from the gland, through the integument, to the exterior where, on account of its low surface tension (as postulated by Yonge (1932, 1938) for the decapod Crustacea), it spreads out to form a thin continuous layer over the chitin.

In the case of the 'salivary' glands it was shown that the glands on the maxilla in an operculate barnacle are partly concerned with the rejection of excess particles in the sea-water, since, (a) they secrete cuticular material, (b) they become partly exhausted in an animal kept in a thick carmine suspension, and, (c) there are no other glands available to produce the entangling threads which are of cuticular material. Nevertheless their main rhythm coincides with the production of cuticle at the moult, and the cuticle is thickest in the vicinity of the glands and becomes thinner with increasing distance from them. They

must, therefore, be regarded as tegumental glands, and the misleading name 'salivary' should be discarded. In addition the name maxillary gland applies to the excretory organ of the whole of the Crustacea, so that for the tegumental glands on the fused second maxillae a new name must be used. Labial tegumental glands is probably the most suitable. For the glands behind the labium, or in the bases of the first three pairs of cirri, a general term 'suboesophageal tegumental gland' would serve to indicate their position close to the suboesophageal ganglion.

During the examination of the labial and suboesophageal tegumental glands of *Balanus perforatus* certain differences were noticed and attributed to the additional function in the labial glands of assisting in rejection. The suboesophageal glands were similar to the labial glands of *Lepas hilli* (where suboesophageal glands are absent), and an examination was therefore made to see if the finely granular secretion and nozzle-shaped pores of the labial glands occurred in any of the available pedunculate cirripedes. A negative result after the examination of sections of *Lepas anatifera*, *Scalpellum scalpellum*, and *Lythotrya valentiana* indicates that rejection may not occur in pedunculates, and this habit in the operculates may be associated with their abundance between tide marks where the water is frequently muddy. That the glands are incompletely adapted to this accessory function is indicated by their rapid exhaustion and their inability to continue to supply material for rejection over a period corresponding to the normal time of immersion of a barnacle at or below half-tide level.

Yonge (1932) has shown that the cuticle is much harder than the chitin, and the thick cuticle near the mouth of the Decapoda has been attributed to the need for a strong surface layer where the passage of food materials causes wear. A similar thickening of the cuticle near the mouth is present in the cirripedes and can be attributed to the oral position of the main tegumental glands.

7. SUMMARY.

1. In the Cirripedia the exo-skeletal integument consists of a thick layer of chitin with a thin superficial layer of cuticle.

2. The cuticle agrees in properties with that of the Decapod Crustacea, and is also secreted by tegumental glands.

3. Unicellular or compound glands, here first described, secrete the cuticle of the outer surface of the peduncle and capitulum; the labial and suboesophageal ('salivary') glands secrete that of the surface of the mantle cavity.

4. In the Operculata the labial glands also secrete between moults, the cuticular material serving to entangle excess material which enters the mantle cavity and so assist in its rejection. The restriction of this accessory function to the Operculata may be correlated with their abundance in frequently turbid waters between tide-marks.

5. The cement is identical in properties with the cuticle and the cement glands are regarded as modified tegumental glands.

6. In the Pedunculata all the gland-cells regenerate after secretion. The labial and suboesophageal glands secrete only at the moult, in the other glands some of the cells are always active.

7. In the Operculata secretion is confined to the moult apart from the secretion produced by the labial glands in connexion with rejection of particles.

8. In the Operculata the cells of the cement glands degenerate after secretion, new cells developing from the walls of the duct.

9. The specializations of the tegumental glands are correlated with the sessile habits of the Cirripedia.

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The Fate of Spermatozoa in the Female Dogfish (*Scylliorhynchus canicula*).

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With Plate 16 and 1 Text-figure.

INTRODUCTION.

THERE is conflict between authors regarding the fate of those spermatozoa injected into the female genital tract at the time of copulation and which are not afterwards used in fertilization. The majority of investigations relate to mammals where the spermatozoa are extremely small and their post-coital history difficult to follow. In part, confusion has arisen as a result of the unwarranted assumption that the spermatozoa of different animals suffer the same fate. Little of the literature is recent, no paper deals with the elasmobranch fish, and none of the authors has given photographic evidence. The present paper describes the post-coital history of the large and distinctive spermatozoa of *Scylliorhynchus* (*Scyllium*) *canicula*. The figures on Plate 16 are untouched photographs, excepting fig. 2 A.

HISTORICAL.

Kohlbrugge (1910), who worked on various mammals, gives full references to earlier works. He states that in the Javanese bat (*Sp.* unnamed) the cells of the uterine mucosa exert a chemotactic influence on the spermatozoa, liberating them from their coagulum and increasing their activity. The vaginal secretion is not chemotactic. The spermatozoa bore into the wall of the uterus at right angles, their tails are lost and the heads may suffer any one of three fates. (1) The cells of the uterine mucosa secrete a digestive enzyme around them turning them into food

vacuoles which resemble the watery-looking nuclei of some of the mucosa cells. (2) The heads of the spermatozoa bore into the nuclei of the mucosa cells and there disintegrate forming nuclear granules. (3) They bore right through the uterine mucosa into the connective tissue beneath and there penetrate and fuse with some of the small nuclei of the connective tissue cells. This, says Kohlbrugge, accounts for the dark appearance of some of these nuclei in stained preparations.

Kohlbrugge also describes dark conical masses among the cells of the uterine mucosa which he assumes to be spermatozoa before undergoing the first of these fates.

According to Kohlbrugge innumerable spermatozoa are to be found in the uterine glands of women shortly after copulation.

Kohlbrugge's findings agree essentially with those of Philippi (1909) on the viviparous Teleost *Glaridictyus*. An account of fusion between the heads of spermatozoa and the nuclei of certain cells in the wall of the uterus of the guinea pig is given by Guieysse (1908). On the other hand Sobotta (1910, 1920) accuses Kohlbrugge of poor technique and faulty observation and disagrees with him on every point. Sobotta states that in rodents there is no boring of spermatozoa into the uterine glands or mucosa, nor does he believe that it occurs in any animal, and he adds that dead spermatozoa either leave per vaginam or are absorbed by leucocytes. He dismisses Kohlbrugge's story of nuclear fusion as a figment and asserts that the 'food vacuoles' resembling the watery nuclei which Kohlbrugge describes, are, in reality, invading leucocytes. Sobotta adds that, in the ejaculate, one sees a great destruction of spermatozoa, and it looks as though only those spermatozoa are phagocytosed which are still motile and happen to bore into a leucocyte. In this way, he says, the leucocytes may protect the uterine wall from invasion by spermatozoa.

Popa and Marza (1931), working on the dog and guinea pig, state that copulation is followed by strong desquamation of epithelial cells. The only fate of the spermatozoa described by them is their wholesale phagocytosis, throughout the whole lumen of the female genital tract except the vagina, by invading

leucocytes. As a result, only an occasional spermatozoon would survive to reach a position favourable to fertilization.

METHOD.

Scylliorhynchus canicula has been used exclusively. The fish were caught at Roscoff (Brittany) in April 1939. Thirteen oviducts were removed, each from a different fish. Three of the fish (Nos. 1-3) were immature. Five (Nos. 4-8) were adult but 'non-pregnant', and five (Nos. 9-13) were 'pregnant', with egg cases in utero.

Representative portions of each oviduct were fixed in the following fixatives:—(1) Formol Zenker, (2) Formol Müller, (3) Carnoy, (4) Bouin, (5) Heidenhain's Susa. Transverse and longitudinal sections were taken through the oviduct at various levels, and also through the cloaca. They were stained in (1) Heidenhain's iron haematoxylin, (2) Mallory's triple stain, and (3) a modification of Papanicolaou's stain for vaginal smears (1933).

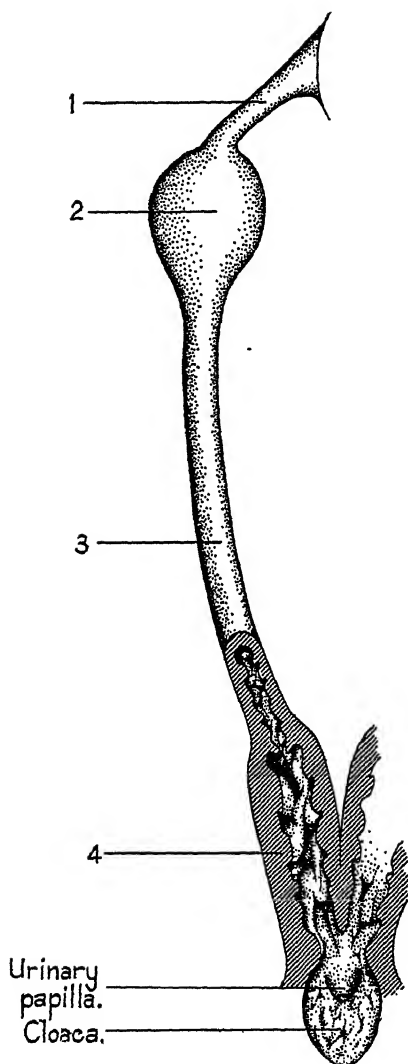
THE OVIDUCTS.

The oviducts of the dogfish are divisible each into four parts, Text-fig. 1: (1) a narrow anterior thin-walled portion, between the ostium and the oviducal gland, lined by ciliated columnar epithelium; (2) the oviducal gland similarly lined; (3) a long thick-walled region lined by ordinary columnar epithelium and which expands suddenly into (4) the 'uterus', which is wider, very thick walled, and lined by stratified epithelium. The two 'uteri' open side by side into the cloaca which is also lined by stratified epithelium.

The 'uterine' epithelium is thicker in a 'pregnant' fish than in a 'non-pregnant' one, otherwise there is little change in the structure of the oviducts during 'pregnancy'. In the lumina of the 'uterus' and cloaca there is detritus of variable content.

THE SPERMATOZOA.

The spermatozoon of the dogfish has been adequately described by Retzius (1902). It is characterized by its length (160 μ), and by the spirally twisted head.



TEXT-FIG. 1.

Right oviduct, part of 'uterus' of left oviduct and cloaca of dogfish, *Scylliorhynchus canicula*. (Uteri and adjacent oviduct on right side, opened to show lumina.) Ventral view. $\times 1$.

OBSERVATIONS.

The Cloaca.

Certain fish in each of the three groups (immature, adult 'non-pregnant', 'pregnant') exhibited vacuoles in the epithelium lining the cloaca (Table 1). The fact that these vacuoles are present in immature fish, and also that in mature fish no spermatozoa were ever found in them or anywhere else in the cloacal wall, shows that vacuoles in the cloacal epithelium are not related to absorption of spermatozoa and therefore do not correspond to anything described by Kohlbrugge. When present, they contain no spermatozoa but blood corpuscles, chiefly red, in all stages of degeneration. They are formed first at the base of the epithelium from the capillaries of the subepithelial connective tissue. Each vacuole is formed by the rupture of a superficial capillary, and the passage of one or more corpuscles into the epithelium where they become incorporated, at once, in a clear vacuole resulting from the activity of the epithelial cells. In company with these cells they pass towards the surface and are sloughed off with the cells of the superficial epithelium. This accounts for the presence of blood in the cloacal detritus of certain fish. Those fish without vacuoles in the cloacal epithelium showed no blood in the cloacal detritus. There is no increase in the proportion of leucocytes to red corpuscles, either in the capillaries of the connective tissue or in the vacuoles. If any digestion of the corpuscles occurs within the vacuoles, it is not completed by the time the vacuoles reach the surface of the epithelium.

The Cloacal Detritus.

Apart from faecal matter the cloacal detritus may contain three things: (1) blood corpuscles of both kinds but chiefly red, (2) cells sloughed from the superficial epithelium, (3) spermatozoa. Of the ten adult fish, spermatozoa were found in the cloacal detritus of four, three 'non-pregnant' and one 'pregnant'. No spermatozoa were found elsewhere in the cloacal region of any fish. The number of epithelial cells was very variable. Generally very few were present, but they were never entirely absent from an adult fish, and occasionally they were abundant.

The number of epithelial cells bore no relation to the sperm content of the cloacal detritus, so that desquamation does not follow copulation as in the dog and guinea pig (Popa and Marza, 1931).

The 'Uterus'.

The vacuoles in the 'uterine' epithelium were rare and confined to three adult fish, two 'pregnant' and one 'non-pregnant'. One of the 'pregnant' fish (No. 9) had numerous vacuoles and some of them contained spermatozoa. Each such vacuole contained from one to many spermatozoa in various stages of degeneration and sometimes, in addition, degenerating blood corpuscles. In no other fish were spermatozoa found in the vacuoles of the 'uterine' epithelium. On the other hand, one of the 'non-pregnant' fish (No. 6) possessed many spermatozoa embedded in the 'uterine' epithelium, although vacuoles were absent.

The 'Uterine' Detritus.

The 'uterine' detritus was never abundant. Desquamation of the epithelium is apparently slow and continuous. In this and the absence of faecal matter the 'uterine' detritus differs from the cloacal detritus. Spermatozoa were found in the 'uterine' detritus of fish Nos. 6 and 13. One fish only (No. 6) contained an abundance of spermatozoa in the cloacal detritus. It follows that this fish had copulated recently, as the sperms deposited in the cloaca would soon either have been expelled with the faeces, or they would have ascended the oviducts. This accords with the fact that in this fish alone spermatozoa were found entering the 'uterine' epithelium and also ascending the upper oviduct. In fish No. 9 spermatozoa were found in vacuoles in the 'uterine' epithelium and not elsewhere. It follows that this fish had copulated earlier than fish No. 6, or, in other words, that the spermatozoa burrow into the 'uterine' epithelium first, and that their enclosure within a vacuole is a later event. From the evidence supplied chiefly by these two fish the following history of the spermatozoa has been worked out.

The spermatozoa burrow head first into the 'uterine' wall at

TABLE I

Number of fish	Condition of fish	Cloaca				'Uterus'			
		Epithelium		Detritus		Epithelium		Detritus	
		Vacuoles	Sperm content	Blood corpuscles	Epi-thelial cells	Sperm content	Epi-thelial cells	Blood corpuscles	Sperm content
1	Immature .	-	-	-	-	-	-	-	-
2	? Adolescent	+	-	+	+	-	-	-	-
3	Immature .	-	-	-	-	-	-	-	-
4	'Non-pregnant'	+	-	+	+	-	+	-	-
5	'Non-pregnant'	+	-	+	+	-	-	-	-
6	'Non-pregnant'	+	-	+	+	-	-	-	+
7	'Non-pregnant'	+	-	+	+	+	-	-	-
8	'Non-pregnant'	+	-	+	+	-	-	-	-
9	'Pregnant'	+	-	+	+	-	-	-	-
10	'Pregnant'	+	-	+	+	+	-	-	-
11	'Pregnant'	+	-	+	+	-	-	-	-
12	'Pregnant'	+	-	+	+	-	-	-	-
13	'Pregnant'	+	-	+	+	+	-	+	+

The symbol + denotes 'present', and is repeated in cases of abundance.

The symbol - denotes 'absent'.

The symbol * denotes 'sperms not in vacuoles'.

right angles to it (fig. 1, Pl. 16). Eventually the long heads lie entirely embedded among the cells of the 'uterine' epithelium (figs. 2 and 2 A, Pl. 16). Their passage is intracellular. They may afterwards turn in any direction, but on the whole they maintain their original direction. Once embedded the very delicate tails of the spermatozoa could not be found, but it is impossible to confuse their long spiral heads with the nuclei of the surrounding cells. The cells in contact with the heads of the spermatozoa break down and form a clear globular vacuole around them which is gradually carried to the surface of the epithelium and so shed (fig. 3, Pl. 16). A single vacuole may contain one or more spermatozoa. It may, in addition, contain degenerating blood corpuscles (fig. 4, Pl. 16). As with the blood corpuscles, the degree of degeneration of the spermatozoa within the vacuoles is very variable.

This fate of the spermatozoa accords with the first of the three fates as described by Kohlbrugge. It disagrees with the findings of Sobotta. There is, however, no justification for Kohlbrugge's assumption that the vacuoles are food vacuoles.

One isolated spermatozoon was found which had burrowed right through the 'uterine' epithelium and was lying in the superficial connective tissue beneath. Its state of preservation was good.

Of the other two fates accorded to the spermatozoa of the bat by Kohlbrugge, neither occurs in the dogfish. Nor were the dark conical masses encountered, which he describes in the 'uterine' epithelium and which he supposes to be spermatozoa before secretion of the vacuole.

The Upper Oviduct.

It has already been stated that the oviduct anterior to the 'uterus' is lined by columnar epithelium. About five millimetres of the oviduct adjacent to the 'uterus' is subject to a heavier invasion of spermatozoa than is the 'uterus' itself. In places clusters of spermatozoa can be seen lying within the columnar epithelium, chiefly towards its base (fig. 5, Pl. 16). It is noteworthy that a very high proportion of the spermatozoa arrange themselves parallel to the length of the oviduct. There

is, however, no question of this representing an alternative mode of ascent, as no spermatozoa are found within the walls of the oviduct further up. The spermatozoa commonly pass right through the epithelium and lie in the connective tissue just beneath. They do not bore into the nuclei of the connective tissue cells. No vacuoles are found in the columnar epithelium, so that spermatozoa in it and in the connective tissue are presumably phagocytosed.

Conclusion.

Popa and Marza (1931) explain the mass slaughter of spermatozoa in the lower oviduct as a prophylactic to peritoneal infection; the spermatozoa acting as vectors of bacteria. In their opinion it is only an occasional spermatozoon that survives. In the dogfish, where the oviducal gland is also a receptaculum seminis (Metten, 1939), such a device would defeat its own ends. As a matter of fact the oviducal lumina of fish No. 6 contained abundant spermatozoa anterior to the zone of destruction. Also, the average sperm content of the oviducal glands of all adult dogfish is quite high (Metten, 1939).

The peculiar behaviour of the spermatozoa of certain invertebrates is worthy of mention here. Manton (1938) has shown that in *Peripatopsis* the male deposits the spermatophores anywhere on the outside of the female, and that the spermatozoa burrow right through her body to reach the ova in the ovaries. Here, most of the spermatozoa are absorbed by the young oocytes which turn them into yolk. None of the spermatozoa is consumed by leucocytes. Apparently the spermatozoa are necessary to the normal development of the ova. In the female bug *Cimex*, most of the spermatozoa are normally absorbed by 'resorptions Organe' (Abraham, 1934). Grove (1925) found that in the earthworm, *Lumbricus terrestris*, sections of the spermathecal wall, which is lined by columnar epithelium, show spermatozoa embedded in the free ends of the epithelial cells. In his opinion it is the mucin or some other protein material which attracts the spermatozoa, and by which they are nourished during their long stay in the spermatheca. Presumably such spermatozoa are capable of future emergence without loss of viability, in which

case this process has no relation to that which entails their destruction, as found in the female genital tract of vertebrates. But Grove produces no evidence that the spermatozoa which invade the wall of the spermatheca do, in fact, live.¹

It could be argued that the destruction of spermatozoa in the oviduct is a device for killing the less viable spermatozoa. However, the ability to penetrate the thick 'uterine' epithelium is proof to the contrary; such spermatozoa must possess a high viability.

Until further investigation throws more light on the problem of sperm absorption in the female genital tract, it is here suggested that the epithelium concerned produces two secretions, one nutritive to the spermatozoa and capable of diffusion out of the cells into the lumen of the oviduct, the other hostile to the spermatozoa and incapable of such diffusion. The concentration of the nutritive secretion being higher within the cells than outside them, would explain the motive for invasion. The killing by the hostile secretion, of those spermatozoa which had forced their entry, would be a device for protecting the oviducal wall. Whilst the presence of such secretions remains hypothetical as yet, this theory does account for the peculiar and uneconomic behaviour of the spermatozoa in the female genital tract.

SUMMARY.

1. References are given to the literature concerning the fate, in various vertebrates, of spermatozoa in the female genital tract.

2. In the dogfish, penetration of the oviducal wall by spermatozoa does occur. It is confined to the 'uterus' and about five millimetres of the oviduct anterior to it. The latter suffers a denser invasion than the 'uterus'. No spermatozoa invade the cloacal epithelium.

3. Epithelial vacuoles are found in the 'uterus', where they

¹ It is well known that in certain Turbellaria the spermatozoa may be injected at copulation into the body and make their way to the site of fertilization through the parenchyma; while in Hirudinia the spermatozoa may be deposited on a special surface area and reach the ova along specialized tracts of tissue.

may or may not contain spermatozoa, and in the cloaca, where they do not. Any of the vacuoles may contain red and white blood corpuscles.

4. Vacuoles containing blood corpuscles account for a continuous 'uterine' bleeding on a small scale.

5. Desquamation of the 'uterine' epithelium is slow and continuous, that of the cloacal epithelium intermittent and heavy. Desquamation is not related to the time of copulation.

6. Digestion of spermatozoa and blood corpuscles within the vacuoles may be very incomplete.

7. The spermatozoa which enter the columnar epithelium near the 'uterus', frequently pass right through and into the superficial connective tissue. No vacuoles are produced in the columnar epithelium. Presumably all such spermatozoa are phagocytosed.

8. No fusion occurs between the heads of the spermatozoa and the nuclei of any of the cells of the oviducal wall, as described by Kohlbrugge.

9. The proportion of spermatozoa destroyed in the lower oviduct is not sufficient to prevent them ascending the upper oviduct in large numbers.

10. A theory is advanced to account for the facts concerning the destruction of spermatozoa in the oviduct.

The author is indebted to Professor J. Gray for providing research facilities in the Zoology Department, Cambridge. The photographs are the work of Mr. P. Mumby.

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EXPLANATION OF PLATE 16

BICls, blood corpuscles, degenerating; *ColEpi*, columnar epithelium; *ConT*, subepithelial connective tissue; *L*, lumen of oviduct; *HdSp*, head of spermatozoon; *StrEpi*, stratified epithelium; *Sp*, mass of spermatozoa; *Vac*, vacuole.

All figures of *Scylliorhinus canicula*.

Fig. 1.—Transverse section of the stratified epithelium of the ‘uterus’. A spermatozoon is entering the ‘uterine’ epithelium. A short length of the tail of the spermatozoon is visible near the head. $\times 450$.

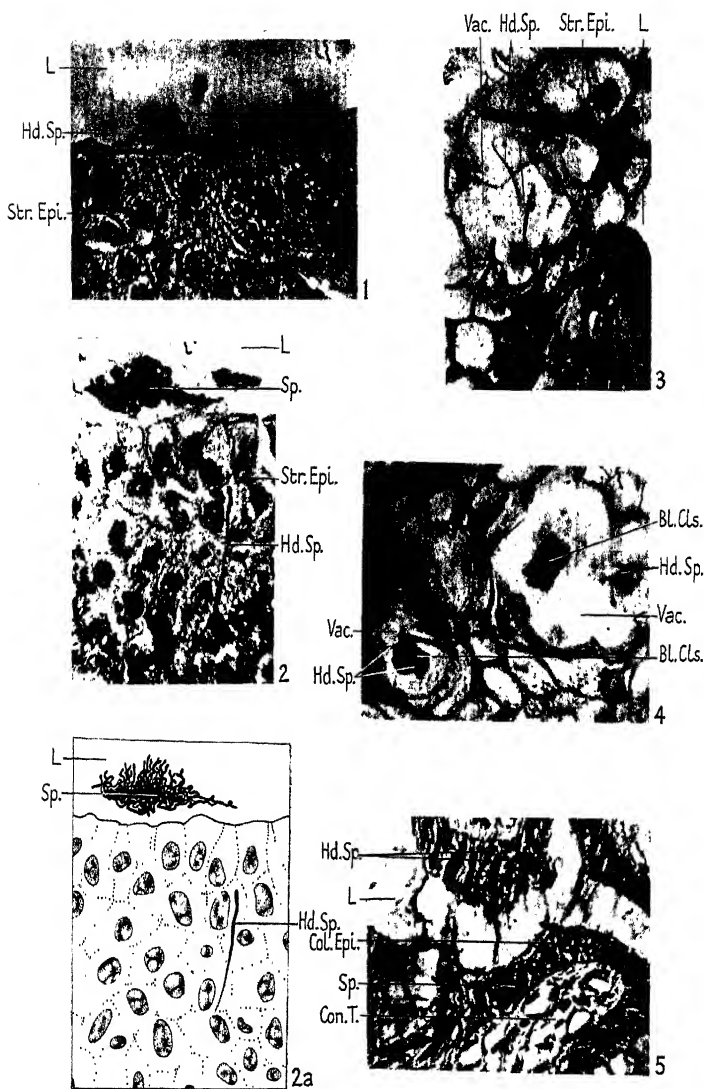
Fig. 2.—Similar section of the ‘uterus’. A spermatozoon has recently entered the epithelium, and its head is visible completely embedded in the cells of the epithelium. $\times 450$.

Fig. 2 A.—Pen and ink drawing of Fig. 2.

Fig. 3.—Similar section of the ‘uterus’. The heads of two spermatozoa are visible in the epithelium, and the epithelial cells in their vicinity are beginning to break down and form a vacuole round them. $\times 450$.

Fig. 4.—Similar section of the ‘uterus’. Two vacuoles are seen in the epithelium. Each vacuole contains a number of degenerating spermatozoa and also some degenerating blood corpuscles. $\times 450$.

Fig. 5.—Transverse section of part of the oviduct adjacent to the ‘uterus’. Lying in the columnar epithelium is a mass of spermatozoa. The heads of other isolated spermatozoa are seen in transverse section (they are lying parallel to the length of the oviduct). $\times 450$.



***Amoeba lescherae* (nov. spec.)—Its morphology, cytology, and life-history.**

By

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With Plate 17 and 6 Text-figures.

SOURCE OF MATERIAL.

In the spring of 1938 we put some well washed water weeds,¹ which had been stored for more than a week in a moist atmosphere, into a glass trough containing Glasgow tap-water, for the purpose of obtaining protozoa (Taylor, 1920).

On 29 July, 1938, an examination of the macerated material revealed a few adult amoebae which looked superficially like *Amoeba proteus* Y. Wheat grains were added to the culture to supply pabulum as we have found from previous experiments that the decaying vegetation did not supply sufficient organic substance (Taylor, 1919, p. 179) for the needs of the protozoa. The amoebae were comparatively numerous on 22 September, 1938, 20 to 30 specimens being visible in the contents of a solid one inch square watch glass when viewed under the low power of a Greenough binocular. The amoebae were adhering firmly to the weeds either because the ectoplasm possesses adhesive properties or because the creatures were burrowing among the plants for the rotifers on which they were feeding. A brief examination of the amoebae made us resolve to keep the culture for future examination. When we next looked at the culture on 24 December, 1938, the amoebae did not seem to be thriving. The nutritive spheres (Pl. 17, *N.S.*) were large, and the wheat grains were not decomposing with sufficient rapidity to supply the needs of the ciliates and flagellates required as food

¹ Sent from Wales for Botanical teaching.

organisms by the amoebae.¹ We therefore strained the whole contents through a daphnia net, and added a mixed diet of flagellates and small ciliates together with some wheat grains to the 'filtrate'. On 25 April, 1939, this filtrate (culture 26680 in the Field Book) was in magnificent condition: the parent culture (i.e. the debris left in the daphnia net) was, on the other hand, overrun with green paramecia and showed no amoebae. But by dint of adding flagellate food organisms, and for reasons that will be evident later on, this parent culture recovered, and from these two initial cultures we have grown abundant supplies of this amoeba which thrives under laboratory conditions.

METHODS OF CULTIVATION.

The procedure described above, namely the periodical addition of a few wheat grains to a well established culture, is satisfactory when it is desirable to preserve a more or less natural environment for the amoebae, and it has the advantage of providing 'stock' material which is always available for the inoculation of new cultures. But for an intensive and prolonged study necessary to elucidate the life-cycle, more prolific sources had to be obtained. The first question is one of food organisms upon which the amoebae makes great demand. It was found necessary to cultivate these separately. Flagellates and ciliates from the parent culture were isolated and cultivated for this purpose, a constant supply of these being always at hand. For the purpose of viewing the cultures quickly both under the Greenough binocular and under the ordinary microscope it has been found advisable to grow the amoebae in Petri dishes. With the help of a $\frac{1}{7}$ -inch water-immersion lens the amoebae contents of all ages can be examined in situ alive and without any detriment to them. To start a new culture Glasgow tap-water was poured into a 3 to 4 in. diameter Petri dish. To this was added three to four boiled wheat grains and about 5 c.c. of a rich 'flagellate' culture. Amoebae picked out separately from 'stock' with as little of the parent culture water as possible were then

¹ To anticipate what will emerge later in the course of this account, a frequency of 'depression' periods is characteristic of *Amoeba lescherae*.

inoculated into the prepared Petri dish. The food organisms thus get a start and are consequently always in excess of amoeba demands. In a month a flourishing culture of young and rapidly dividing amoebae results. In course of time this optimum condition gives place to a 'depression' period. A strict routine must accordingly be observed to keep up supplies of this amoeba at the particular stage in its life-history required for investigation. Sub-cultures must be prepared regularly.

IDENTIFICATION OF THE AMOEBIA.

Its Classification.

It was a natural sequence of the events narrated above to compare and contrast the newly discovered amoeba with *Amoeba proteus* Y which it resembled in many ways. Gradually, from a study of the adult we came to the conclusion that it was not *Amoeba proteus* Y. We would have arrived much more quickly at this decision if we had had at the outset the developmental stages, for these are very different in the two species. After consulting the literature we concluded that the species had not been described before, and decided to name it *Amoeba lescherae* (to honour the memory of the Founder of Notre Dame College in Glasgow—Mary Adela Lescher, S.N.D.). A great deal of confusion exists concerning the identity of large, free-living amoebae. In his 'Taxonomy of Amebas' Schaeffer (1926) has endeavoured to introduce greater precision of nomenclature. In this work (Schaeffer, 1926, p. 42) he re-introduces the Linnaean generic name *Chaos* for the large, free-living amoebae of which Rosel's '*Der Kleine Proteus*' (= *Chaos chaos*) is the type. At the time of the publication of the above work Schaeffer had not decided whether '*der Kleine Proteus*' was synonymous with Muller's *Amoeba proteus*, or whether he should give the name to Wilson's *Pelomyxa carolinensis*. After further study (Schaeffer, 1936-7) he has decided in favour of Wilson's *Pelomyxa carolinensis* which becomes *Chaos chaos*. Having examined specimens of the *Amoeba proteus* which we had cultivated continuously since 1918 Schaeffer concluded that it

was the same as the American one, so we adopted his nomenclature and referred to our type *Amoeba proteus* Y (Carter, 1919) as *Chaos diffluens*. Since then Schaeffer has expressed doubts as to the validity of his identification, and Mast and Doyle (Singh, 1938) say it is not the same as the one they made use of. Many authors refer still to our *Amoeba proteus* as *Amoeba proteus* Y. We shall therefore retain this name here.¹

The amoebae of the genus *Chaos* are large and form pseudopodia that are sub-cylindrical, blunt, and filled with granular endoplasm throughout. A well marked characteristic of these amoebae is that they bear conspicuous longitudinal ridges and grooves in the ectoplasm. Crystals and nutritive spheres are among the cytoplasmic inclusions. *Amoeba lescherae* belongs to the genus *Chaos*, and if Schaeffer's nomenclature be finally adopted it will become *Chaos lescherae*.

Other sources of supply of *Amoeba lescherae*.

Amoeba lescherae has now been obtained in pond dippings sent from the south of England. We have not yet discovered it free in Scotland. We are indebted to Dr. Slack of the Zoology Department of Glasgow University for sending to us specimens of an amoeba found by Mr. A. Mackinnon at the bottom of an aquarium containing *Lebistes* (commonly called guppy). These turned out to be a whole developmental series of *Amoeba lescherae*, ranging from newly hatched individuals to young adults.

Morphology of *Amoeba lescherae* (Pl. 17).

In describing an amoeba it is essential to state the stage in the life-history of the individual, and the condition of the culture from which the specimen has been taken. As will be evident after a perusal of this paper, the appearance of any amoeba

¹ Because of war conditions we have not been able to find out to what conclusion the American workers have come as to the identity of the European and American *Amoeba proteus* = *Chaos diffluens* (Schaeffer). Hyman (1940) maintained that zoologists in general do not accept the changes in nomenclature proposed by Schaeffer.

changes with its age, and with the character of the food it has been taking. The essential features of structure are best seen in an amoeba which has just attained the adult stage, which moves freely when transferred to a slide, and which is feeding freely.

When a successful Petri-dish culture of from one to two months old is viewed over a black background under a Greenough binocular, four different conditions of the amoebae can be picked out.

(1) *Creeping Forms*.—There are large numbers of varying form creeping on the bottom of the dish. Some are more or less limax-shaped, in which individuals the longitudinal folds show up well and give considerable height to the amoeba. Sometimes the available cytoplasm is spread out into great antler-shaped individuals. Often the whole amoeba forms the two arms of a V: these arms may be very close together or widely separated.

(2) *Radiating Floating Forms*.—Other amoebae in the culture are floating, their pseudopodia being more or less radiate.

(3) *Large Spherical Forms*.—Conspicuous, out-sized, spherical individuals more opaque than the rest are to be found, though not in large numbers.

(4) *Mammilated Spherical Forms*.—Spherical individuals with a mammilated surface are to be found on occasion. These latter are the amoebae about to undergo fission, referred to in this paper as 'division spheres'. The fission of the cytoplasm may be viewed by confining one's attention to one such 'division sphere'.

Now, a good criterion for 'standardizing' the amoeba to be described is to examine the rate of fission. Young, typical adult amoebae of all species divide regularly, the rate differing for different species. In a flourishing culture *Amoeba lescheræ* divides once in 24 hours at a temperature of 70° F. If a creeping individual be examined in situ with a $\frac{1}{4}$ -inch water immersion, the longitudinal folds in the ectoplasm (a diagnostic character of the genus *Chaos*) are easily seen. The length may attain to 500 to 600 μ . It is therefore slightly smaller than *Amoeba*

proteus Y. Large spherical individuals, when transferred to a slide and allowed to creep, attain a size of $525\ \mu$ by $600\ \mu$. These individuals are most often bi-nucleate, but may contain three or four nuclei. The average diameter of the spherical forms is $350\ \mu$. Multinucleate individuals occur also in cultures of both *Amoeba proteus* Y and *Amoeba discoides*, the maximum number of nuclei being greatest in *Amoeba proteus* Y. The explanation of their occurrence is, however, still obscure. They only begin to appear when a culture has attained its optimum activity. Levy (1924, 1928) proves that in *Amoeba proteus* Y the condition is brought about by the failure of the cytoplasm to divide after mitosis, and that the viability of the cells is lowered by the multinucleate condition. Chalkley (1931), commenting on the great variety of size of amoebae in any one culture, shows that volume is correlated with the number of nuclei present, and confirms Levy's conclusion that the mortality rate is highest in the large, multinucleate individuals.

The radiate, floating individuals will also creep if transferred to a slide. In old specimens stuffed with cytoplasmic contents the rate of movement is slowed down. These senile individuals are smaller than young adults and much more opaque in reflected light. They are found in cultures that are more than three months old. What the forces are that bring about the marked diminution of the water content of these ageing amoebae has not yet been discovered (cf. Parsons, 1926).

In a youngish creeping *Amoeba lescheræ* the pseudopodia vary in number from three to seven. In every case the pseudopodia are blunt and of the direct locomotion type (Schaeffer, 1926); the endoplasm flows to the tip, the granular contents then cascade over, their place being taken by advancing granules. The pseudopodia formed when a large multinucleate individual is adopting the creeping condition radiate from the centre, sometimes resembling a dense fringe. The narrow diameter of these radiating pseudopodia recalls those of the developing amoeba (Text-fig. 5 c and d) (see later). When, however, the pseudopodia are developed from one area of the circumference only, they are necessarily broader. Often in

addition to the main pseudopodia in a young adult several other pseudopodia of less width occur, the ends of which are bifid, and antler shaped specimens are common. Young individuals move rapidly forwards. Sometimes the pseudopodia become 'two-storeyed'. As the individuals grow older there is a greater tendency for the amoeba to send out pseudopodia in all directions. After having gripped the substratum and moved definitely in the same direction as its axis with the help of two or three main pseudopodia, it suddenly thrusts its pseudopodia upwards towards the coverslip, or radially, in either case loosening itself and floating away. After some time it may return to the creeping habit. On account of this characteristic *Amoeba lescheriae* is not so suitable for teaching purposes, the floating amoebae being easily carried away out of the field of view.

Crystals (Pl. 17, c).—Penard (1902) describes multinucleate amoebae which 'avaient tous leurs cristaux sans exception . . . rectangulaires, avec arêtes et angles parfaits, et apparaissent selon toute apparence au système quadratique'. The crystals of *Amoeba lescheriae* are very characteristic and distinguish this species from others of the genus *Chaos*. They are square prisms varying in size according to the age of the individual and attaining a maximum diameter of 2μ . When they are viewed obliquely they bear a superficial resemblance to the coffin-shaped crystals of *Amoeba proteus* Y. More prolonged observation at different angles reveals their true character, the crystals being rolled about by the streaming endoplasm so that their true shape may be appraised. We have not discovered any change in shape such as sometimes occurs in *Amoeba proteus* Y, when conspicuous, rectangular slab-like plates appear among the normal crystals. We have associated the formation of these anomalous crystals with an unhealthy condition of the individual possessing them, but the true significance awaits investigation. The chemical composition of the crystals in amoebae is a subject that has attracted many American writers, but the technical difficulties attending the elucidation are very numerous. Luce and Pohl (1935) conclude that they consist of calcium chlorophosphate. They do not,

however, state the species of amoeba upon which they worked. We have been unable to perform a chemical analysis of the crystals of *Amoeba lescherae*.

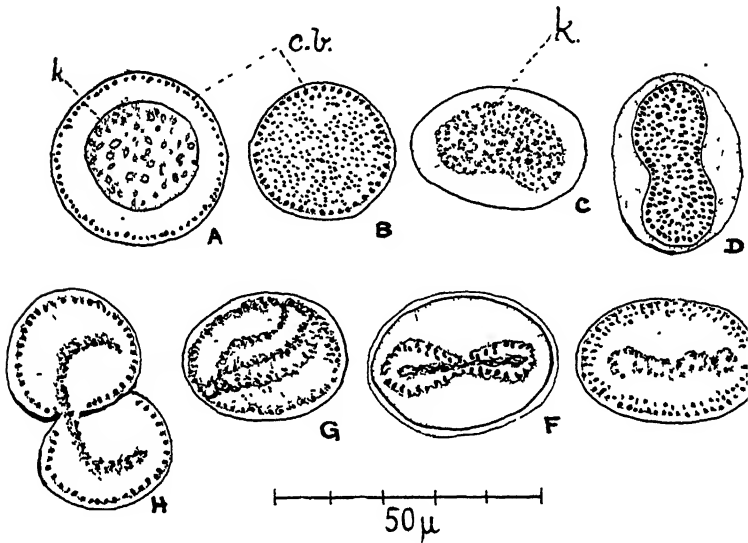
Nutritive spheres (Pl. 17, *N.S.*) are present. They vary in size according to the age of the individual and the character of the food organisms. In *Amoeba lescherae* fed on *Chilomonas* the nutritive spheres develop quickly. They attain a maximum diameter of $7\ \mu$. In young individuals their diameter is $1\ \mu$ to $2\ \mu$ (see later).

Although translucency in amoebae viewed over a black background varies with age and physiological condition, *Amoeba lescherae* is not so opaque as *Amoeba proteus* Y. The shapes assumed by the contrasted amoebae also differ. Much more of the total bulk of the cytoplasm is concentrated in the pseudopodia of *Amoeba lescherae* than in *Amoeba proteus* Y. As will be seen later this contrasting behaviour of the adults is anticipated in the immature developing individuals (cf. D and E, Text-fig. 5, p. 315).

Nucleus.

Normally there is present one nucleus, discoid in shape, which in the living animal is subjected to the streaming movements of the endoplasm (Taylor, 1930). Hence an observer sees alternately the circular or the elliptical outline of the nucleus that is being studied. The elliptical outline remains longest in view. In a young adult the change over from one view to the other takes place quickly. The size of the nucleus depends upon age; the longest axis varying from $30\ \mu$ to $52\ \mu$ in the adult. In the centre of the nucleus is a disc denser than the surroundings which we shall refer to as a karyosome (Text-fig. 1, *k*). This is connected up with a peripheral layer of regularly arranged blocks (Text-fig. 1, *c.b.*) normally just under the nuclear membrane. Only in newly metamorphosed amoebae is this typical structure exhibited (Text-fig. 1 *A*) for reasons that will transpire later. A voluminous nuclear sap fills up the rest of the nucleus. The shape of the karyosome changes continually as does also its consistency. In some views it looks like a band stretching across the nucleus (Text-fig. 1 *E*). Some-

times the karyosome extends to the periphery, and is very evenly mottled, when it can only be distinguished from the peripheral portion by the larger size of the granules in the latter (Text-fig. 1 B). At times the peripheral granules seem to be



TEXT-FIG. 1.

The resting nucleus of *Amoeba lescherae*.

A, Newly metamorphosed nucleus, clear space between karyosome (*k*) and peripheral chromatin blocks (*c.b.*). B, Karyosome extending to peripheral chromatin blocks. C, D, No chromatin blocks in periphery. E, Karyosome in 'elevation', chromatin blocks in two or three layers. F, Karyosome and periphery blocks biconcave in shape. G, Complicated nucleus. H, Nucleus so constricted as to give the appearance of division into two.

many layers deep (Text-fig. 1 E). This is especially the case when the band-shaped karyosome is displayed. A fuller description of the nucleus will be available later in the section devoted to stained preparations. Seen against a black background the living nucleus appears to be almost as transparent as the ectoplasm.

Contractile Vacuole.

There is one contractile vacuole and, when nearing the diastole, it is roughly the same diameter as the nucleus, which latter is typically situated just anterior to it. It first becomes conspicuous in the central region of the amoeba, is displaced towards the posterior end as it increases in size and there evacuates very deliberately. The facility with which both nucleus and contractile vacuole may be studied makes *Amoeba lescherae* a useful object for demonstrating these structures to elementary students.

Excretion.

Excretion apart from the contractile vacuole can be best studied with the help of a $\frac{1}{4}$ -inch water-immersion lens. Excreta, including crystals, are apparently gathered into a large vacuole, where violent commotion is set up, the contents being swirled around. After a few seconds the excretory mass is violently shot out of the vacuole.

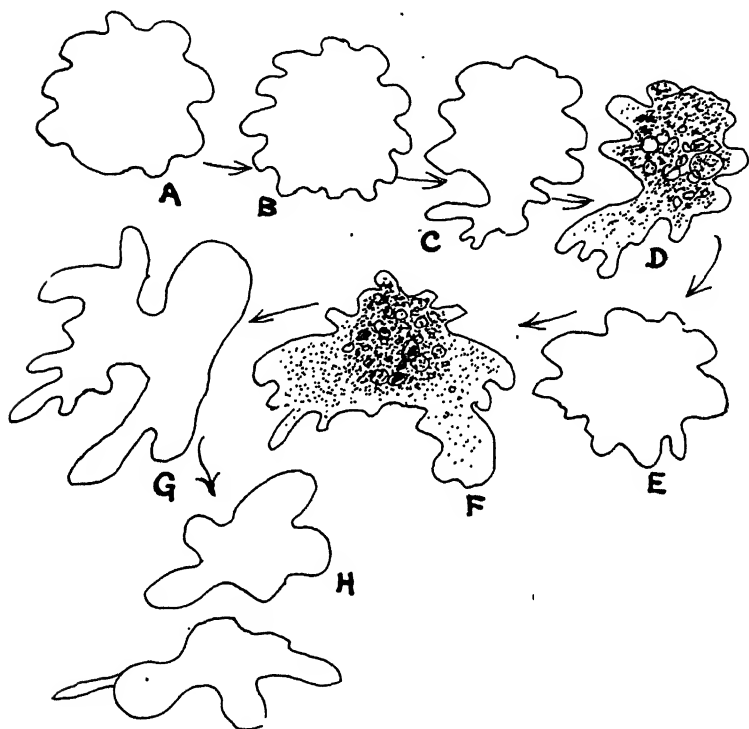
Food.

Amoeba lescherae feeds voraciously on small ciliates, flagellates, and rotifers. It is quite capable of seizing and digesting *Paramecium*. The ingestion of food organisms temporarily disturbs the movement of a creeping amoeba. It would appear as though the protoplasm around a newly formed food vacuole had to remain stationary for a time.

Fission (Text-fig. 2).

Amoebae about to undergo fission are readily identified by their spherical shape and mammilated surface ('division spheres'). The process occupies about 15 minutes. There is nothing to remind one of the cleavage-like furrow of a segmenting cell which is so characteristic an appearance of the fission divisions of *Amoeba proteus* Y. By contrast *Amoeba lescherae* has often little contact with the substratum and can use floating debris as well as the substratum for anchorage during fission (Text-fig. 2). Two daughter sets of pseudopodia are differentiated on either side of the incipient

line of separation and these enable the daughter amoebae to creep away from each other in opposite directions. There is no long, slender thread of protoplasm linking up the daughter amoebae as they separate. The break is clear cut (Text-fig. 2



TEXT-FIG. 2.

Fission of cytoplasm of *Amoeba lescherae*. A and B, 'Division spheres'. C, D, Cytoplasm showing local activity. E, Protruded portions shown in C and D withdrawn. F, Central area very dense; two protruding portions. G, 'Line' of division quite clear. H, Two daughter amoebae: one adhering to debris, the other to the

G, H). In aceto-carmin preparations of 'division spheres' lines of force reminiscent of the spindle fibres of a metaphase nucleus are to be seen on either side of the fission axis. The contrasting

conditions obtaining in *Amoeba lescherae* and *Amoeba proteus* Y described above are confirmed in these studies of the cytoplasmic conditions in the region of the axis of separation of the daughter amoebae. In the latter they extend down the whole dividing amoeba.

Pure Line Cultures are easily procured by the method already described for the Petri-dish cultures, one amoeba only being inoculated into a small Petri dish containing a few organisms for food. If the inoculation is successful, the amoeba divides once in 24 hours and continues to do so. Fresh food organisms should be given gradually. When the culture is sufficiently strong it should be transferred to the definitive Petri dish where wheat grains can be added in the usual way.

Stained Preparations of Resting Nuclei (Text-fig. 1).

Method. Large numbers of *Amoeba lescherae*, fixed in Bouin's fluid and allowed to harden in 90 per cent. alcohol for some hours, were then transferred to centrifuge tubes where they were stained in Ehrlich, differentiated in acid alcohol, dehydrated, cleared, and mounted, in Canada balsam. The appearance of the nucleus is so varied when large numbers are studied that it is best to refer back to a very young adult to procure a 'standard' (Text-fig. 1 A). The karyosome in such a specimen does not wholly fill the centre of the nucleus—the blocks in the periphery stain well in Ehrlich, but a comparison with the prophase chromosomes to be described later shows that these 'blocks' are composite in character, that besides true chromatin there is in them an underlying substrate (= plastin) which is probably chromatin in the making (cf. Taylor, 1924 and 1930). The karyosome presents a variety of appearances. This fact may be appreciated by a scrutiny of Text-fig. 1, where it is seen to be coarsely and unevenly mottled, regularly and evenly dotted with densely staining particles, band shaped, corrugated. Similarly the peripheral blocks may be one (Text-fig. 1 B) or many rows deep (E), or entirely removed from the

nuclear membrane (cf. c, d, e). Sometimes the nucleus is so constricted as to give a false appearance of division (Text-fig. 1).

In a series of studies of spherical amoebae (see p. 299) the diameter of the nucleus in 25 counts averaged $42\ \mu$ and in 5 other counts ranged from $30\ \mu$ to $56\ \mu$. In most binucleate types the diameter of each nucleus averages $42\ \mu$. As in *Metachaos discoides* (= *Amoeba discoides*) the peripheral blocks are comparatively larger than in *Amoeba proteus* Y, the achromatic framework supporting them being finer and less conspicuous. The reaction of the nuclear membrane to staining varies greatly. In some cases the nuclear membrane is stout and deeply stained; in others so thin as to be hardly discernible. We have suggested elsewhere that the amoeba nucleus lies in a vacuole. There is a good deal of evidence for this hypothesis.

MITOSIS.

Introductory.

The first investigator to describe mitosis in the large, free-living amoebae was Dr. Lucy Carter (1913). Mitosis in the living *Amoeba proteus* was seen by Chalkeley (1934). The mitosis of *Amoeba dubia* (= *Amoeba proteus* Pall. of Carter) was investigated by Dawson, Kessler, and Silberstein (1935), and subsequently Liesche (1938), although he does not state clearly which species he used (probably *Amoeba dubia*), published a lengthy account of nuclear division. Whether our *Amoeba proteus* Y is identical or not with the *Amoeba proteus* used for a very full investigation of mitosis by Dawson, Kessler, and Silberstein (1937) we cannot be sure. We hope at a later date to go into the question.¹

¹ Many writers stigmatized the material used in 'Nuclear Divisions of *Amoeba proteus*' (Taylor, 1923) as pathological. Since we still have large supplies of amoebae the direct descendants of the cultures we used for that investigation and since we have given material to most Universities in the British Isles and to friends in New Zealand, Australia, Europe, and the U.S.A. we must state that we ascribe the phenomena there described to causes other than pathological conditions. Old adults tend to have nuclei that are easily contorted into S-shaped structures. As already mentioned, sometimes the constrictions are so extreme as to give an appearance of actual division into two daughter nuclei (cf. Text-fig. 1 e). To reiterate what has already been said, typical nuclei are to be found

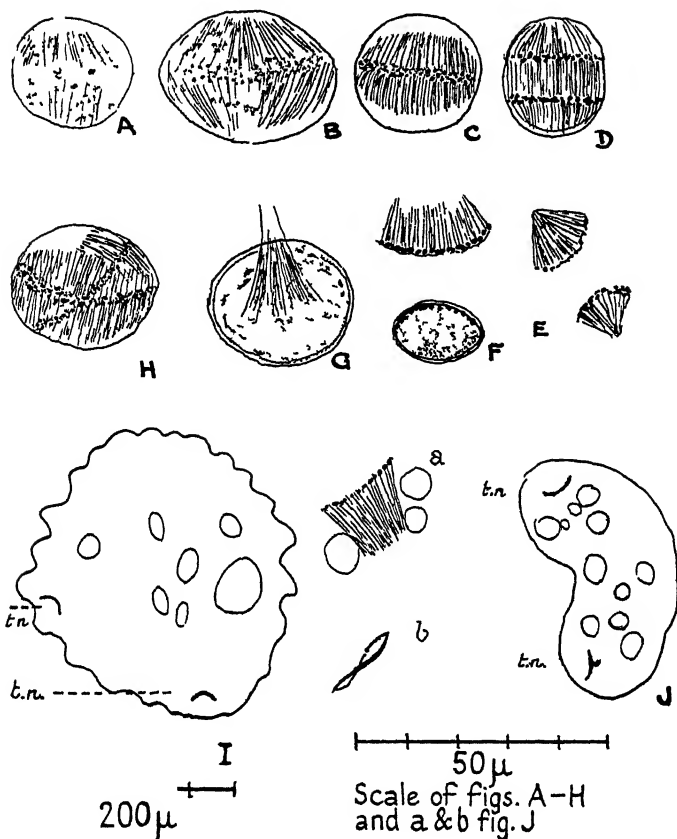
Stages in Mitosis.

Stages in Mitosis were obtained by two procedures.

Method I.—The amoebae, which often adhere to the wheat pabulum and the hyphal threads that sometimes emanate from it in an 'optimum' culture, having been gently teased off the grains, the latter were picked out of the Petri dish and the whole of this very luxuriant culture was then fixed, stained, and mounted (see under Nucleus, p. 306). A careful search through the slides thus produced was made and the mitotic stages studied.

Method II.—A modification of the cooling-heating effect on division of the nucleus described by Dawson, Kessler, and Silberstein (1937) was adopted. Flourishing Petri-dish cultures were kept cool by enveloping them overnight in a damp cloth. About two hours before the preparations were made the cultures were placed under an electric lamp which brought them to a temperature of 80° F. 'Division spheres' were then picked out, transferred to a slide, fixed, stained, and made permanent by the irrigation method.

Ehrlich's haematoxylin was used throughout, as this has been checked with Feulgen's reagent (Taylor, 1929). The amoebae containing mitotic (Taylor and Hayes, 1942) stages were easily discovered under a $\frac{2}{3}$ -inch objective, and studied with a Zeiss 2-mm. objective. The first indication that mitosis is about to occur is seen in the 'blocks' which, as already explained, contain in addition to true chromatin a substrate of material, which, while clearly distinguishable from the purely achromatic material, does not react to Ehrlich as does pure chromatin, and is only faintly stained by Feulgen's reagent (Taylor, 1929). We interpret this substrate as the preliminary phase of the fully differentiated chromatin (cf. Plastin, Taylor, 1924). At certain stages of metabolism it is quite impossible to distinguish only in young adult amoebae. To a lesser extent the same contorted and constricted nuclei are to be found in *Amoeba lescherae*. Faulty interpretation of appearances rather than pathological material is responsible for our conclusion (Taylor, 1923; Hayes 1938). It is important to realize that these complicated nuclei are found in amoebae procured from freshly gathered pond-dippings just as they are under cultural conditions. They are accordingly not due to laboratory environment.



TEXT-FIG. 3.

Mitosis in *Amoeba lescherae*.

A, Early prophase. B Early metaphase. C, Metaphase. D, Anaphase to early telophase. E, Telophase. F, Late telophase, one of the daughter products showing early signs of reconstruction of daughter nucleus. G, Reconstruction of resting nucleus: spindle fibres still present. H, Multiple division—two metaphase plates. I, Outline of a stained amoeba showing position of the telophasic nuclei (*t.n.*). J, Ditto, *a* and *b* enlarged drawings of respective telophasic nuclei.

true chromatin from this underlying matrix (= plastin). At the onset of mitosis these blocks become linked up with each other, looking like strings of beads which, apparently under the

influence of some force, become more or less meridionally arranged inside the nuclear membrane. Gradually the nucleus loses its staining capacity, and this fact makes the detection of the nucleus at this stage difficult (Text-fig. 3 A), especially in a whole mount of a well-fed amoeba. The chromatin, never voluminous, now stands out in contrast to the residual material which becomes converted into spindle fibres (Text-fig. 3 B and C). These become orientated around two poles in a barrel-shaped nucleus, the small, numerous chromosomes migrating to the equator where they stand out clearly, the whole looking like a typical metaphase. The spindle fibres do not always reach the two poles at metaphase; there seems to be a definite cavity there, containing no fibres. Later the fibres grow out to the poles. There are no obvious centrosomal structures.

Anaphase.—In early anaphase the chromosomes become massed together and begin to lose the brilliancy of their staining capacity (Text-fig. 3 D).

Telophase.—The daughter products look like two cones as they separate (Text-fig. 3 E). Individual chromosomes become less easily distinguishable. By this time the nuclear membrane of the mother nucleus has disappeared, but a new nuclear membrane to each daughter product is secreted. True chromatin is sparsely distributed in the achromatic elements inclosed in the nuclear membrane (Text-fig. 3 F). Remains of spindle fibres sometimes persist for a time (G). Telophasic stages (of the two daughter nuclei) are readily seen in the amoeba before the actual fission of the cytoplasm takes place (see Text-fig. 3 I and J, *t.n.*) when it may be observed that the chromatin material still remains for a time much more conspicuous on one half of the circumference than on the other (Text-fig. 3 F).

Reconstruction of the Daughter Nuclei.

The reconstruction of the daughter nuclei begins just before the cytoplasm of the respective daughter amoebae becomes independent. The nuclear membrane having been secreted, the available chromatin becomes broken up into irregularly sized masses which disperse among the achromatic elements now being differentiated in the daughter nuclei. The bulk of

the daughter nucleus increases on account of the increase in nuclear sap. These new nuclei look much like empty vesicles at this stage, and can easily be identified in a crowd of stained individuals by the fact that there is so little 'solid' matter in the nucleus.

Multinucleate Divisions (Text-fig. 3 H).—Examples of a double mitosis are by no means rare in luxuriant cultures. It appears then that the multinucleate condition is brought about by normal mitosis. As in the uninucleate condition the metaphase and anaphase stages are produced inside the nuclear membrane.

Agametogony.—Emission of chromidia, according to Minchin (1912), is due to the exhaustion of the nucleus. We have now come to the conclusion that my term 'chromatin blocks' (Taylor, 1924) is equivalent to chromidia.¹

Good preparations of the stages in agametogony may be obtained by the following method: Select a culture about three months old, one that has not been allowed to deteriorate because from it sub-cultures have been regularly made, and one in which immature amoebae can easily be perceived under the $\frac{3}{8}$ -inch objective. Take adults which have not lost their power of adhering to the substratum and which are of maximum size and possessed of large nutritive spheres. Fix these in acetic alcohol for twenty-four hours and mount in aceto-carmine.

An examination of such slides will reveal the presence of chromidia in the cytoplasm as well as stages in the formation of the agametes.

¹**Chromatin Blocks.**—The name used for the small masses of nuclear material regularly arranged under the nuclear membrane of many amoeba nuclei. They contain chromatin material on a basis or framework of plastin. In agametogony the chromatin blocks migrate into the cytoplasm.

Chromidia.—According to Minchin (p. 65) the term 'Chromidien' was coined by Hertwig to denote extra-nuclear grains of chromatin. A difference in the use of the term by subsequent authors induced Minchin to differentiate, in chromidia, between the true chromatin which he called chromidiosome and the underlying substance which was not chromatin. Hence I follow him in using chromidia for 'Chromatin Blocks' when describing agametogony.

Chromosome is used in its generally accepted sense of the thread-shaped bodies which make their appearance in the prophase of mitosis.

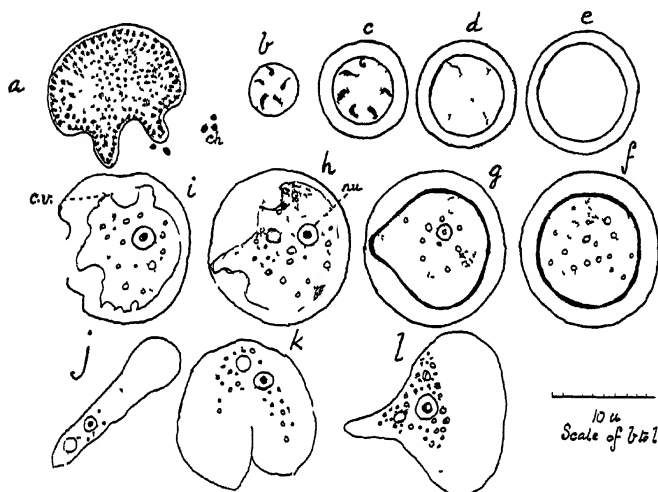
Permanent preparations for a study of agametogony were obtained by fixing large quantities of adult amoebae in Bouin's fluid.

These fixed amoebae were stored in 90 per cent. alcohol for five months when they were again returned to Bouin to make sure that all the mitochondria were dissolved out, as were the crystals. These amoebae were stained in Ehrlich for twenty hours and differentiated in acid alcohol, cleared, and mounted. The nuclei were characterized by deep constrictions which occurred irregularly. Little clusters of chromidia were to be seen escaping from some of the nuclei (Text-fig. 4). The nuclear membrane showed great diversity in thickness, being entirely absorbed in some cases. The nutritive spheres were not easily distinguishable from the rest of the cytoplasm, the pellicle (see paragraph on Cytoplasmic inclusions) giving the impression of being so much extended as to appear transparent and just ready to allow the contents to be merged into the general cytoplasm. Small chromatin blocks (= chromidia) were dispersed throughout the cytoplasm, these being in various stages of metabolism as evinced by their staining reactions. Another batch of specimens, also characterized by the number and size of their nutritive spheres were stained in Ehrlich and light green when the chromidia were seen to be well developed. These chromidia form the rudiment of the nuclei of the agametes. There seems to be no doubt that the nutritive material of the spheres is used up in the formation of the agametes which are quite clearly distinguishable from the former. In spite of much adverse criticism we still maintain this thesis of the reproductive cycle (Taylor, 1924).

Chromidia gather around themselves an envelope of cytoplasm which is separated from the cytoplasm of the agamont by a definite wall (Text-fig. 4, *a-e*). The outer rim of this newly formed cytoplasm becomes thickened and so the cyst is two-walled (Text-fig. 4, *f*). As development proceeds the chromidia lose their staining capacity so that eventually no distinction between chromatin and cytoplasm can be detected, the whole becoming a spherical and not easily stained mass (Text-fig. 4, *e*).

Method of obtaining free, living, Amoeba lescheriae cysts.

(1) From a pure-line culture (four months old) make a series of preparations in which several large, adult amoebae (agamonts) are mounted in their own culture medium. Place these



TEXT-FIG. 4.

- a*, Nucleus of agamont with chromidia escaping into cytoplasm. *b-e*, Stages in the formation of the agamete. *f*, Encysted agamete from the bottom of a culture. *g*, Pear-shaped encysted amoeba. *h*, Outer cyst wall perforated. Encysted amoeba with pseudopodium, contractile vacuole, and nucleus. *i*, Amoeba ready to escape from cyst. *j*, Excysted amoeba, limax shaped. *k*, Rounded amoeba, formed from *j* by a large, lateral pseudopodium. *l*, Later stage, amoeba now feeding. *ch*, chromidia (= chromatin blocks). *cv.*, Contractile vacuole. *nu*, Nucleus. *a* is drawn to same scale as Text-fig. 1.

in a damp chamber. After a varying period of time encysted amoebae may be found in the debris caused by the disintegration of the agamonts.

(2) Make several mounts of the debris from the bottom of a four months old culture. Keep these in a moist chamber.

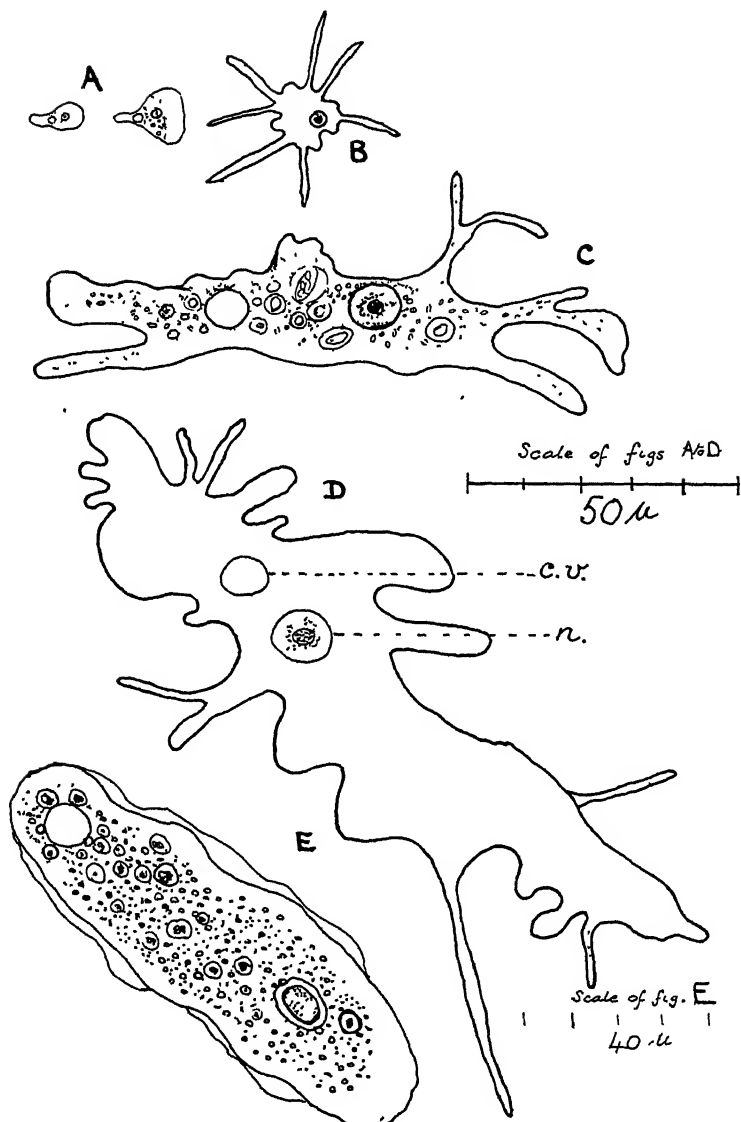
Cysts about $13\ \mu$ in diameter may be recognized by their obvious amoeba-like contents and their bluish tinge. In the course of from three to twenty-one days young amoebae hatch out.

Description of Cysts, Excystation. The average diameter ($13\ \mu$) of *Amoeba lescherae* cysts is a little greater than those of either *Amoeba proteus* Y or *Amoeba discoides*. The outer and inner walls are at first close together. As the cyst develops the outer wall becomes perforated in one place, a conclusion which is reached because (1) floating particles can now indent the outline, (2) a large number of cysts show pear-shaped contents, the apex of the pear being thickened (Text-fig. 4, *g*) and in close contact with the outer wall as though secreting there a digestive fluid. The mass inside the perforated, but otherwise intact, cyst wall shows no sign of movement for several subsequent hours. It, however, gradually shows signs of differentiation (Text-fig. 4, *g*). At last the inner wall of the cyst becomes 'dissolved' and a finger-like pseudopodium protrudes (Text-fig. 4, *h*). At the same time a contractile vacuole begins to function and the nucleus becomes clearly distinguishable (Text-fig. 4, *h* and *i*). Eventually the outer cyst wall is abandoned and a very active limax-shaped amoeba emerges (Text-fig. 4, *j*). Gradually it begins to feed copiously on small food particles, its cytoplasm spreading out to give it a triangular outline (Text-fig. 4, *l*). A very marked characteristic of the ectoplasm of developing *Amoeba lescherae* is a facility to curl up, or fold over, a facility which the creature shows very markedly at this stage. Newly excysted amoebae often round off and cease to move, in which condition they might be mistaken for encysted individuals.

Development (Text-fig. 5).

For a long time we neglected to study the small¹ sized amoebae which turned up very frequently in *Amoeba lescherae* cultures in view of the fact that at least two species of *Mayorella* are frequent inhabitants of amoeba cultures made from pond collections. When, however, small amoebae appeared in pure-line cultures we realized that they belonged to

¹ $20\ \mu \times 10\ \mu$.



TEXT-FIG. 5.

Figs. A-D. Development of *Amoeba lescheriae*. Fig. E, Immature *Amoeba proteus* Y. c.v., Contractile vacuole. n, Nucleus.

the life-cycle of *Amoeba lescherae*. As already indicated these developmental stages are in marked contrast to those of *Amoeba proteus* Y (Text-fig. 5, cf. c and d with e).

For purposes of description it will be easier to begin with individuals visible with the high power of a Greenough binocular (Text-fig. 5 d). These occur in great numbers in a flourishing culture, are very transparent, usually longer than broad, are floating, or creeping along the weeds or on the bottom of the Petri dish, and are possessed of pseudopodia of varying diameter. Transferred to a slide and covered with a cover-slip they settle down to creep, when their pseudopodia are seen to be in marked contrast to *Mayorella* of any species, for they direct the movement of the amoeba, and are seen to be of the Chaos type, i.e. the endoplasmic granules run to the tip of the blunt pseudopodia. In the more slender pseudopodia the granules are in a single row, in the blunt ones they are more numerous. The ectoplasm is voluminous, but does not flow as readily as in the adult. The size of the endoplasmic granules varies according to the nature of the food organisms ingested; coarse if *Chilomonas* has been the food; fine, if small ciliates have been eaten. As in the adult the pseudopodia form tiers and are very numerous, the long feeler-like ones being sometimes used for anchorage. At all stages of its development *Amoeba lescherae* has the power of feeding on organisms nearly as large as itself. In very young specimens we have observed the substance of the amoeba, looking like a mere ring encircling an outsized food vacuole. In such creatures the nucleus is plainly visible and the contractile vacuole functions normally.

Stages which can only be found with the help of a $\frac{2}{3}$ -inch Objective.

These are more radiate in form and look highly vacuolated because of the relatively large diameter of the numerous food vacuoles. When feeding on ciliates these young are especially transparent, the granules in the endoplasm being extremely fine. When the amoeba is packed with food vacuoles the radiate pseudopodia consist almost wholly of ectoplasm and the observer has to wait until digestion is effected before the

flow of the endoplasmic granules into the pseudopodia can be witnessed. *Amoeba lescherae* can capture food while floating. '

Stages which require a $\frac{1}{4}$ -inch Objective for their Identification.

These have already been described in the paragraph on excystation. Their development, which is slow, may be traced by cultivating them on a slide in a damp chamber.

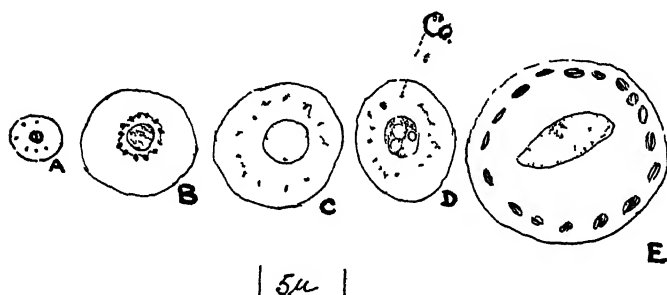
In any one culture the amount of time that elapses from one state of 'depression' (p. 297) to the next is 3 months. *Amoeba lescherae* thus grows more quickly than *Amoeba proteus* Y. It also thrives better at a higher temperature. In a 'natural' culture, i.e. a culture with the same environmental conditions as those which obtain in nature, the vicissitudes of these developing amoebae are numerous. When adults are replaced by the agametic cysts, rotifers, paramecia, stylonychia, &c., have a chance of increasing rapidly. The constant currents set up by the action of the strong cilia possessed by these organisms swirl about the newly hatched and small delicate amoebae and give them no chance to feed quietly. Hence if adults of mixed ages are not abundantly present the culture must be cleared of these large infusorians or very few amoebae will attain maturity.

As developing amoebae grow older their cytoplasmic contents gradually accumulate, thus giving them a more robust appearance. This, coupled with the increase in volume, renders them more opaque. Not, however, until the adult condition is reached can definite crystals and nutritive spheres be recognized. Crystals of the characteristic shape are only found in well developed adults.

Nucleus of the developing *Amoeba lescherae*.

The nucleus is always easily seen in the living amoeba. It consists (Text-fig. 6 c) of a central mass of more solid material, the karyosome, surrounded by an area of comparative transparency which is, in turn, separated from the cytoplasm by a nuclear membrane. This general description could be applied

to the developing young nuclei of *Amoeba proteus* Y and *Amoeba discoides* (Hayes, 1938) as well as to members of the *Mayorella* genus. Hence it requires time to distinguish one from the other. A characteristic of the nuclei of *Amoeba proteus* Y and *Amoeba lescherae*, which can be seen easily in stained preparations, is the presence of a circle or collar of material (Text-fig. 6 B, C, and D, Co), denser



TEXT-FIG. 6.

Development of nucleus of *Amoeba lescherae*. Stages A-E.

In E the 'collar' is indistinguishable from the nuclear membrane, and shows large 'blocks'.

Co, 'collar' = achromatic network between karyosome and nuclear membrane.

than the nuclear sap and finely beaded, lying between the karyosome and the nuclear membrane. This is absent in the genus *Mayorella*. The 'collar' is sometimes very near the karyosome, at other times it is nearer the nuclear membrane. Sometimes the 'beads' can easily be counted. This structure is the rudiment of the achromatic network which supports the peripheral 'blocks' of the adult nucleus. As the nucleus grows older this ring gradually approaches and becomes permanently associated with the nuclear membrane. The consistency of the karyosome of the developing nucleus varies at different times (cf. C and D, Text-fig. 6): sometimes it is clearly mottled, at other times almost homogeneous. The flowing endoplasm, too, seems able to deform the nucleus temporarily.

As already stated the developing nucleus of *Amoeba proteus* Y has a very similar build. Hence a knowledge of the behaviour of the living cytoplasm and its reaction to fixatives is a very necessary adjunct to the diagnosing of young amoebae in mixed cultures and in distinguishing their respective nuclei. The absence of the 'collar' in *Mayorella* species, and the presence of a broad band of radially striated structure apparently strengthening the nuclear membrane enables the nuclei of this genus to be identified.

Although the nucleus of the developing *Amoeba lescheriae* with its absence of chromatin blocks and the presence of a collar appears to be very different from that of the adult, on closer inspection these differences are seen to be less real and more superficial than deep-seated when the two are compared. Even in adults the achromatic network, with its chromatin blocks, is often separated from the nuclear membrane (Text-fig. 1 c, d, and f), and a varying consistency of the karyosome is likewise a characteristic of the adult.

CYTOPLASMIC INCLUSIONS.

1. Nutritive Spheres.

Nutritive spheres have already been referred to in the foregoing account. Further study of these and their reactions to stains reveals their structure. Each consists of a central mass of granule-free substance enclosed in a pellicle which sometimes gives a reaction for glycogen (cf. Appendix, Taylor, 1924, and 1939). It is possible to crack open these nutritive spheres by gently tapping the cover slip of the slide on which the amoebae have been mounted, or by draining off the water slowly. In these cracked spheres the pellicle can easily be distinguished by its irregular outline. Permanent preparations of these whole or disrupted nutritive spheres may be made as follows. Pick out from five to ten *Amoeba lescheriae* in which the nutritive spheres are fully developed and plentiful. Treat the slide with methyl green (in acetic acid). The nutritive spheres now stand out by their brilliant green colour. Irrigate with a 90 per cent. alcoholic solution of chlorazol-black. The pellicles now stain

purple. Irrigate with cellosolve, clear in xylol, and make permanent with Canada balsam. The purple rim of pellicle contrasts with the green of the interior. Pressure at an early stage of this operation, as already explained, cracks the nutritive spheres. If adult *Amoeba lescherae* be fixed in acetic alcohol and then treated with aceto-carminc the nutritive spheres become very conspicuous, as does the vacuole in which they are situated. The protoplasmic lining of the vacuole is pale pink. Enveloping the slate-blue coloured pellicle is an area of deep red varying in thickness according to the age of the nutritive sphere. The substance of the nutritive sphere is unaffected by the carmine.

2. Crystals. .

These have already been described in the foregoing account, p. 301 (see also below, p. 321).

3. Neutral Red Staining Particles.

These were demonstrated by two methods:

(1) Amoebae were placed on a slide under a cover slip and allowed to adhere before being irrigated with neutral red.

(2) Amoebae were placed in a solid 1 inch square watch glass with as little of the culture fluid as possible before the neutral red solution was added. It was found necessary to use a very dilute solution. One drop of a 1 per cent. aqueous solution of neutral red added to 100 c.c. of water gives a very pale pink tinge to a solution which will stain some particles in one hour, and many more if the amoebae are left in it all night.

A solution consisting of two drops of 1 per cent. neutral red in 100 c.c. of water was found more convenient. After an hour in this solution a great many granules were stained red. These were of extremely small size and in many cases collected together into little masses in one area of the amoeba, sometimes posterior in position, sometimes lateral. Amoebae live quite happily and healthily and behave normally in a solution of this concentration for two or sometimes nearly three days. After that they draw in their pseudopodia and die gradually. The nucleus, and the

nutritive spheres of these dead amoebae are then stained by the neutral red solution, which thus acts as a normal stain.

Effect of Neutral Red on Food Vacuoles.

The food vacuoles are affected by the very weakest concentration of neutral red solution almost immediately. After 15 to 20 minutes every food vacuole becomes coloured, and there is a large range of colours in the respective vacuoles passing from yellow to deep red. The contents of these latter vacuoles are not evenly stained red throughout, some patches being deeper red than others. More prolonged contact with the reagent, however, produces a change, the light stain becoming a deep red, the deep red becoming almost black. These colour varieties and changes are probably indicative of the degrees of digestion reached in the vacuole. Singh (1938) suggests that it is 'possible that the neutral-red bodies migrate into the food vacuoles and act as digestive enzymes in *Amoeba proteus* Y as was observed by Volkonsky (1929) in ciliates', but he was unable to see any evidence of this. In the observations made on *Amoeba lescheræ* there were no particles seen in the food vacuole as small as the neutral red bodies, and no concentration of these bodies near the food vacuoles was demonstrated.

Groups of crystals, however, were observed to appear in vacuoles which had a purplish tint in amoebae that had been left overnight in neutral red. This appearance of crystals in the vacuoles is probably brought about by the neutral red.

4. Fat.

Osmic Acid Test.—There was considerable difficulty in obtaining any evidence of the presence of fat in *Amoeba lescheræ* by the osmic acid test. This, of course, may be due to the physiological condition of the amoebae. After many trials with amoebae from various cultures the best results were obtained with large, young, active adults. Some were put into a 2 per cent. solution of osmic acid in a watch glass, others were treated on the slide. No change in colour was observed before the lapse of two days. After five days a large number of small spheres, about 1μ in diameter and scattered evenly

throughout the cytoplasm, were found to have taken on a dark brown, almost black colour. These darkly stained spheres were quite easily distinguishable from the nutritive spheres which latter, in addition to their larger size (about 5μ in diameter), remained uncoloured by the osmic acid. The 2 per cent. osmic acid is an excellent fixative for *Amoeba lecheriae*.

'Sudan IV' Test.—Gatenby (1937) recommends the fixing of smears in formalin vapour before treatment with Sudan IV. We tried this method and also fixed the amoebae in 4 per cent. formalin solution before treating the material with Sudan IV. The results were poor, if indeed there were any results. Finally we tried combining the two operations. A saturated solution of Sudan IV in equal parts of 70 per cent. alcohol and pure acetone (Gatenby, 1937) acts as a fixative. Previous experience had shown that 70 per cent. alcohol is a fixative of cytoplasm and cytoplasmic inclusions in amoeba (Appendix, Taylor, 1924). We therefore omitted the formalin treatment and treated the living amoebae directly with Sudan IV. The fixation was satisfactory, and in 10 to 15 minutes a large number of spheres were found to have taken on a deep red colour. By contrast with the osmic acid method, in which all the spheres were the same size, the Sudan IV treatment shows some large spheres almost as large as the nutritive spheres, though quite distinct from the latter, and in addition small globules. Although the nutritive spheres become stained by the Sudan IV their tinge is orange and the pellicle stands out clearly, so there is no fear of confusing the two as the fat globules do not show anything of the nature of a pellicle.

Nile Blue Test. Vital Staining of Fat.—Although Nile blue is a vital stain, in the case of amoebae it will act as such only in very dilute solutions. In a concentration of 1 part in 1,000 parts of water the amoebae round up at once and though they remain alive in such a solution for a few hours they do not spread or flow. In a concentration of from one-half to one-quarter of this strength they remain normal for several hours creeping about the slide in an expanded form. This condition greatly facilitates examination and correct interpre-

tation. If the amoebae being examined have been feeding heavily on smallish rounded food organisms these latter become deeply stained in a very short time and tend to mask the other cytoplasmic contents. Where the food vacuoles contain only one small rounded food organism they bear a superficial resemblance to nutritive spheres. These latter are, however, not stained by the reagent. Small, evenly and deeply stained fat globules, as well as globules measuring $1\frac{1}{2} \mu$ to 2μ stand out conspicuously in these actively moving amoebae left for some time in Nile blue. The number of fat globules so stained did not seem to be so great as was the case in the osmic acid test.

Other amoebae to be tested were examined on the slide in the culture medium, which also contained flagellate food organisms, in order to ascertain the number and the character of the food vacuoles before staining. They were then irrigated with Nile blue. No reaction was noticed until after twenty-four hours, when globules stained a deep blue were observed both in the food vacuoles and in the immediate vicinity of these, sometimes completely surrounding them, as though the fat was produced as a result of digestion. The particular flagellate food organisms on the slide in these experiments were not affected by the Nile blue.

Mitochondria.

When a living *Amoeba lescheræ* is examined under the high power numerous very small granules, some, if not all of which show Brownian movement, may be seen in the clearer parts of the endoplasm, especially near the ectoplasm and in the angles between the pseudopodia. From repeated observations on their size and position it is obvious that some of these granules are the neutral red bodies while others are probably mitochondria, for according to Singh (1938) these latter show Brownian movement in *Amoeba proteus* Y.

As a test for mitochondria amoebae were fixed in Champy and stained in Heidenhain's haematoxylin. In many cases small bluish black bodies were visible in the cytoplasm while in others of the amoebae thus treated no such bodies could be observed

Janus green B used as a vital staining test for mitochondria

needed great care as all but the weakest, barely tinted, solutions proved lethal. After some hours, sometimes over-night, in these weak solutions of Janus green B numerous small blue granules were visible in the cytoplasm of the amoebae. In one set of experiments the amoebae were left in neutral red over-night and then transferred to Janus green B. As a result, both red and blue granules were seen in the cytoplasm; the blue granules being presumably mitochondria.

Gatenby (1938) states emphatically, on the evidence of work carried out in his department, that the Golgi apparatus is absent in amoeba and therefore no tests were made on *Amoeba lescherae* for it.

Structures surrounding the Contractile Vacuole.

In *Amoeba lescherae*, as in *Amoeba proteus* Y and *Amoeba discoides*, the one large contractile vacuole is always surrounded by a thickened, collar-like band of cytoplasm in which are embedded bodies of an unknown nature. These bodies are sometimes rectangular, sometimes rounded in outline, generally occur in one row, and in appearance and size (about 2μ) are very similar to the chromatin blocks which lie under the nuclear membrane. When the nucleus and the contractile vacuole lie side by side in the living cytoplasm one sees no apparent difference between the chromatin blocks and the spheres around the contractile vacuole.

After fixation by ordinary methods, such as Bouin's fluid followed by staining in borax-carmin, Ehrlich's or Delafield's haematoxylin, the collar is still clearly visible and the contained structures are stained as deeply as are the chromatin blocks of the nucleus: they give the chromatin reaction to Feulgen. This fact suggests that these structures contain chromatin and that their function is concerned with the control of the contractile vacuole.

Metcalf (1926) mentions a group of 'granules resembling in size and appearance the cytoplasmic granules' which surrounds the contractile vacuole of amoeba, but since it is not clear which cytoplasmic granules are meant one cannot be sure that he is referring to the bodies now being described.

Wilber (1942) in his paper on *Pelomyxa carolinensis* has much to say about granules which surround the contractile vacuoles, and which he considers to be like those present in *Amoeba proteus*. He makes it quite clear, however, that the granules he describes are similar in composition to the mitochondria. In all our three large amoebae there are numerous mitochondria scattered throughout the cytoplasm, but these are much smaller than the granules to which we are referring. Moreover, the blocks surrounding the contractile vacuole show no Brownian movement, and in the living amoebae can be distinguished from the mitochondria without the slightest difficulty. In fact they bear not the slightest resemblance in appearance, size, or shape to the mitochondria.

Effect of Centrifugal Force on *Amoeba lescherae*.

A large number of amoebae were centrifuged for one hour in an electrical centrifuge at a speed of 2,500 revolutions per minute. The process was carried out in the culture medium. As a result of the centrifuging the nutritive spheres were massed together centrifugally. These were overlain by the crystals in the midst of which was the nucleus. The contractile vacuole took up a centripetal position while the smaller bodies, and especially the fat globules, remained scattered through the whole cell. Singh (1938) found that after centrifuging, the fat-bodies occupied the extreme centripetal position in *Amoeba proteus*, but he had subjected his specimens for an hour to a speed of 5,000 revolutions per minute. Probably the speed to which *Amoeba lescherae* was subjected was not sufficient to cause the fat-bodies to take up the centripetal position.

To Sir John Graham Kerr for reading the typescript of this Paper, to Professor E. Hindle for the ever open hospitality of his Department, to Dr. Margaret W. Jepps, always ready to place at our disposal her wide knowledge of Protozoa and Literature, we express our warm thanks. Miss Cecily Brown Kelly has bestowed great care on the execution of the original drawing of Pl. 17. We tender her our grateful appreciation of her skill.

SUMMARY.

1. The discovery of a new species of large, free-living fresh-water amoeba is recorded, and a description of the adult and of its reproduction is given. Although provisionally called *Amoeba lescherae*, it may have to be placed in the genus *Chaos*.

2. A method of cultivating it in the laboratory on a diet of small flagellates and ciliates subsisting on a pabulum of wheat grains is detailed. By starting them in Petri dishes pure line cultures are shown to be easily obtainable.

3. The resting nucleus, discoid in shape, has been shown to bear a general resemblance to that of *Amoeba discoides* as well as *Amoeba proteus* Y. Division is mitotic, and occurs once in twenty-four hours under optimum conditions. In contrast to *Amoeba proteus* Y the separation of the daughter amoeba is facilitated by the mechanical support of minute fragments of floating debris.

4. The cytoplasmic contents include square prismatic crystals, nutritive spheres, fat globules, neutral red bodies, and mitochondria.

5. It has been shown that while the adult has a general resemblance to *Amoeba proteus* Y the respective developmental stages of the two amoebae are in marked contrast.

6. Agametogony is seen to begin with the emission of 'chromidia' from the nucleus. These chromidia form the rudiments of the agametes which differentiate into cysts. Excystation is described.

7. Agametogony occurs more frequently in *Amoeba lescherae* than in *Amoeba proteus* Y, the intervals between the 'depression' periods being shorter in the former than in the latter.

8. The development of the nucleus from the newly hatched amoeba to its adult condition has been traced, and the immature nucleus has been contrasted with that of species of *Mayorella*.

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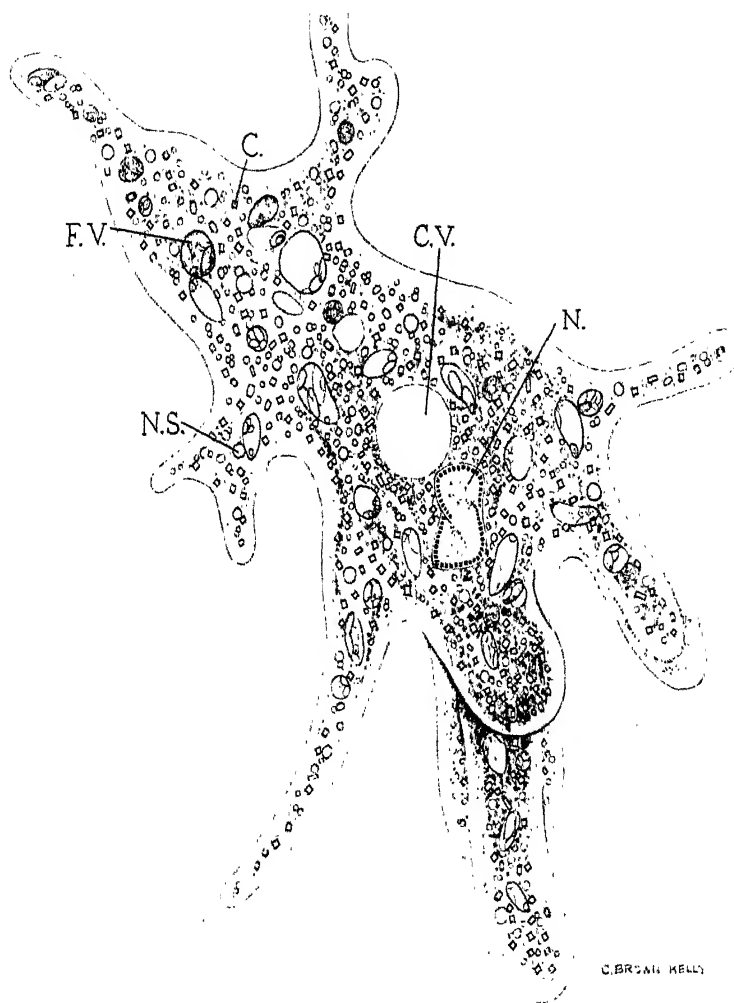
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EXPLANATION OF PLATE 17.

Free hand drawing (not to scale) made from a living adult *Amoeba lescherae* n. sp.

C, crystals; *C.V.*, contractile vacuole; *F.V.*, food vacuole; *N*, nucleus; *N.S.*, nutritive sphere.



Amoeba lescherae

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